

*ANO5 mutations in the Polish limb girdle muscular dystrophy patients: Effects on the protein structure*

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### **Supplementary Materials Legend**

Supplementary Material 1. Description of the patients

Supplementary Material 2. Sequencing data of patients 3-5

Supplementary Material 3. MRI analysis

Supplementary Material 4. Tables

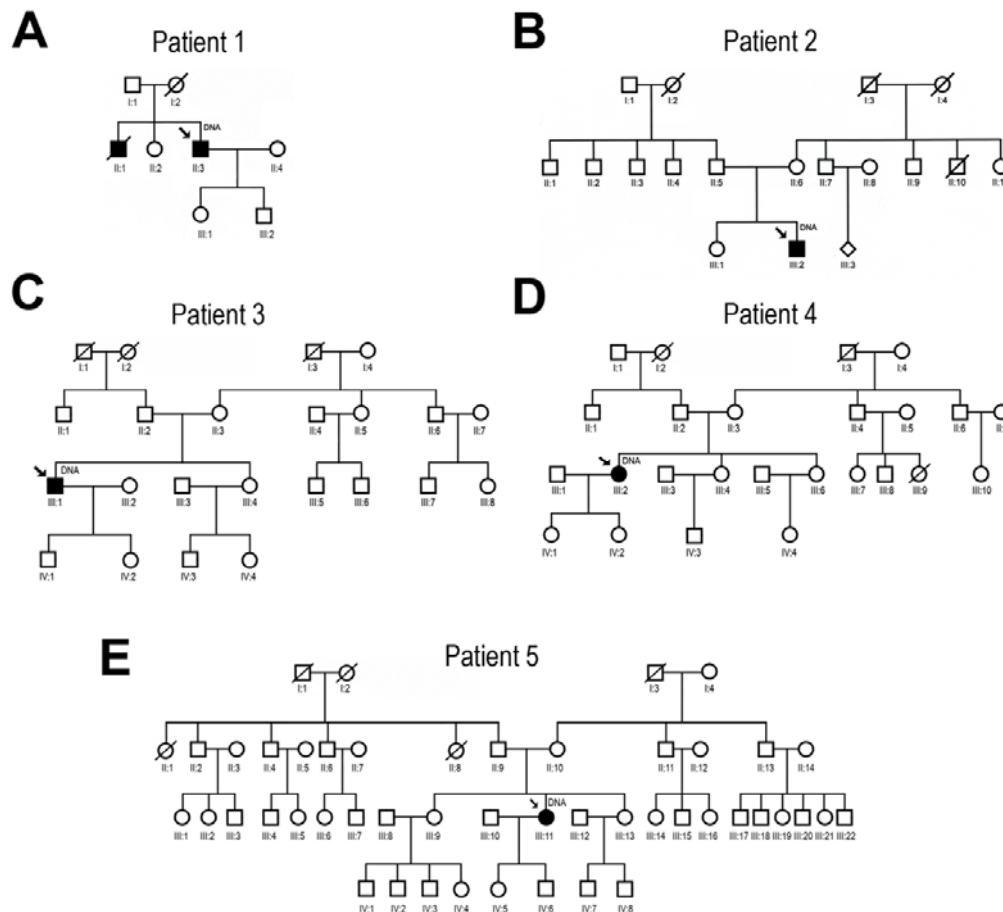
Supplementary Material 5. Secondary Structure Content.

Supplementary Material 6. Principal Component Analyses (Essential Dynamics)

Supplementary Material 7. Motions

## SUPPLEMENTARY MATERIAL 1 - Case Descriptions

### Part I. Pedigrees of the examined families



**Fig. 1.** Pedigree diagrams of LGMD patients. A-E, diagrams of probands' families. Arrows point to probands.

### II. Case Descriptions

**Patient 1:** a 54-year old Caucasian male was admitted with symptoms of LGMD. The proband (Part I A, II:3) was a third child of unaffected, non-consanguineous parents. His deceased elder brother had an undiagnosed neuromuscular disease with gait impairment, while his sister, and his both children (30 and 28 year-old) are clinically unaffected. The proband reported gait problems with a gradual onset in the third decade, moderate myalgia and arthralgia. At 49 years, he underwent cervical spine surgery without improvement, afterwards he noticed slowly progressive weakness and clumsiness of the hands. The proband's medical history included posttraumatic epilepsy treated with Depakine and Lamotrigine, ethanol addiction and abuse, and severe

depression, as well as arterial hypertension, glaucoma and hepatitis B. He was hospitalized twice due to cardiac failure with massive generalized oedema and increased creatinine level of unknown etiology. At 54 years, the proband presented with limb-girdle muscular weakness, mild and asymmetrical in the upper limbs, severe in the lower limbs, with posterior thigh muscles strength most impaired. Biopsy of biceps brachii was then performed. Deep tendon reflexes were absent in lower limbs, there was visible thigh muscle atrophy. There was slight sensory deficit along L4 dermatome in the left lower limb. The proband was able to walk with two crouches with marked knee hyperextension. He required help with sitting up from prone position. Nerve conduction studies revealed axonal-demyelinating mild multifocal involvement of motor and sensory nerves indicating acquired character of peripheral neuropathy. Electromyography showed myopathic changes in the biceps brachii and rectus femoris muscles, while in the distal muscles there were signs of both myogenic and neurogenic process. The level of creatine kinase (CK) was elevated 19 times above normal upper limit. Biceps brachii muscle biopsy revealed advanced dystrophic features. Brain and spinal MR imaging did not show any significant abnormalities. Echocardiography was normal. We have no follow-up of the patient.

**Patient 2:** a male patient was referred to our neuromuscular department at the age of 22 because of myalgia and increased CK level. The patient is the second child of unaffected, non-consanguineous parents (Part IB; III:2). There is no neuromuscular disease history in the family, although slightly increased CK level was found in both parents (less than 1,5 x above the normal upper limit). The patient has a 31-old year unaffected sister, whose CK level is within normal range. At the age of 21, hyper-CKmia of 11000 U/L was detected after increased aminotransferase level had been found in routine blood tests. The patient who was an athlete and a dancer was then asymptomatic. According to available documentation, hypertrophy of quadriceps femoris was noticed, electromyography was normal. In subsequent years the patient developed muscle pain localized mainly in the thighs and shoulders, evoked by exercise and exacerbated a few hours after the exercise. He also reported feeling of increased muscle temperature, trembling or pulsation within the muscles, both muscle and general fatigue, increasing exercise intolerance. He reported infrequent muscle cramps. Exercise tolerance was better in summer months than in winter. Apart from feeling of “heavy legs” during climbing stairs, he did not notice any muscle weakness. On neurological examination generalized muscular hypertrophy was seen, mild scoliosis with right-sided scapular winging, and mild pectus excavatum. Muscular strength was normal except for slight impairment of hip flexors bilaterally. Deep tendon reflexes in the upper limbs and knee reflexes were decreased, ankle jerks were preserved. No contractures were seen. The rest of the neurological examination was normal. CK level fluctuated between 2300-4000 U/L (about 20x

UNL). Electromyography revealed unspecific changes in lower limb muscles suggesting myopathy (motor unit potentials with slightly increased amplitude and shortened duration in vastus lateralis, borderline low motor unit potential amplitude in tibialis anterior muscle; biceps brachii electromyogram was normal). No spontaneous activity during rest was recorded. Motor and sensory nerve conduction velocities were normal. Echocardiography revealed mitral valve prolapse without hemodynamic impairment. 24h electrocardiogram did not show heart rhythm disturbances. Pulmonary function tests were normal. In course of genetic diagnostics, type 2 myotonic dystrophy was excluded and dystrophin gene analysis with MLPA did not reveal deletion or duplication. In a follow-up examination 8 years later, there were no new findings except for mild asymmetry of calf muscles. The patient remains fully ambulatory, able to stand up from the crouch, walk on toes or heels. There is no heart function or respiratory muscle impairment.

**Patient 3:** a 36-year old male patient was referred to the neuromuscular department because of an increased CK serum level (Part I C, III:1). He complained of mild episodic myalgia and muscle cramps. Eleven years before he was diagnosed for a liver disorder because of an increased level of transaminase and bilirubin. Viral hepatitis B and C were excluded. The liver biopsy showed nonspecific changes. The Gilbert syndrome diagnosis was established. At that time CK level was not assessed. One year before hospitalization in our department, the patient was diagnosed due to a high blood pressure and in routine laboratory tests the CK serum level was 15 times above the normal upper limit. The patient is the first child of unaffected, non-consanguineous parents. There is no neuromuscular disease history in the family. The patient was very active in sports since childhood. He is working as a policeman. On neurological examination, he presents with asymmetrical calf and quadriceps muscles hypertrophy, more prominent in the left lower limb. Muscular strength was normal (5/5 in all groups of muscles in MRC scale). Deep tendon reflexes were normal. No contractures were seen. CK level was 10 times the UNL (3108 U/l, N.39-308). Transaminase and GGTP were elevated. Lactic acid curve was normal. Electromyography was normal in biceps brachii and vastus lateralis. However, neurogenic changes were observed in tibialis anterior muscles such as an increased amplitude of motor unit potentials, duration in the upper limit and increased size index (SI). Electroneurography in sensory and motor nerves was normal. Muscle biopsy taken from the left vastus lateralis showed noncharacteristic moderate myopathic changes. Pulmonary function tests were normal. In course of genetic diagnostics, myotonic dystrophy type 2 as well as Pompe disease were excluded. Brain MRI imaging did not show any significant abnormalities. Spine MRI imaging showed L5-S1 discopathy.

**Patient 4:** a 38-year old female was referred to the neuromuscular department because of myalgia and weakness in lower limbs (Part ID, III:2). The symptoms started three months earlier without any triggering factors. At that time, she complained of severe myalgia and lower legs muscle weakness. She could not walk for some days and she used a wheelchair. Initially, she was diagnosed in a local hospital at a neurological department. The CK level was elevated about 8 times above the normal upper limit. EMG revealed myopathic changes. The diagnosis of polymyositis was suspected and steroid therapy (Encorton) in low dose (20mg) was started. Because of poor tolerance and worsening of the symptoms after steroids, the treatment was gradually stopped. Patient was referred to department of reumatology where the diagnosis of polymyositis was excluded. At the age of 34 she underwent a partial strumectomy because of nodular goiter. The patient is the first child among three siblings of unaffected, non-consanguineous parents. There is no neuromuscular disease history in the family. On neurological examination, the patient presented with mild proximal muscle weakness in upper limbs and moderate proximal muscle weakness in lower limbs. All deep tendon reflexes were present and normal. The CK serum level was elevated to 3 times the upper limit. EMG as well as ENG tests were normal. The muscle biopsy showed moderate non-characteristic myopathic changes. No inflammatory cells infiltration were found. Spirometry was normal.

**Patient 5:** a 42-year old female, was referred to our neuromuscular department because of limb-girdle muscle weakness and difficulty in walking (Part IE, III:11). First symptoms appeared at the age of 25 years as weakness of right lower limb with accompanying lumbar pain. After treatment with non-steroid anti-inflammatory drugs, pain subsided but muscle weakness persisted. After a few years, she started to complain of weakness of the left leg resulting in difficulty in running and climbing stairs. At that time she also started to report mild proximal muscles weakness of upper limbs. Two years before admission to our department the CK level was elevated up to more than 20 times of upper normal limits, but EMG was normal. The patient was one of the three children of unaffected, non-consanguineous parents. There is no neuromuscular disease history in the family. On neurological examination the patient presented with mild proximal more than distal muscle weakness of lower limbs (more prominent on the left side), especially in posterior compartment. The patient could not walk on the toes whereas she was walking on the heels without problem. Deep tendon reflexes were normal in upper limbs and weak, but present in lower limbs. The CK serum level was about 9 times of upper normal limits elevated. The EMG revealed myopathic changes in biceps brachii whereas the EMG of vastus lateralis was within normal limit. Electroneurography (ENG) of motor and sensory nerves was normal. Spirometry was normal.

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Supplementary Material 2

Detailed MRI analysis of muscles of patients 2-5. The following parameters were analyzed: muscle volume, muscle fat, fat symmetry and the presence of edema

| Patient/Muscle/Parameter      | Patient 2 |       |              |       | Patient 3 |       |              |       | Patient 4 |       |              |       | Patient 5 |       |              |       |
|-------------------------------|-----------|-------|--------------|-------|-----------|-------|--------------|-------|-----------|-------|--------------|-------|-----------|-------|--------------|-------|
|                               | Volume    | Fatty | Fat symmetry | Edema | Volume    | Fatty | Fat symmetry | Edema | Volume    | Fatty | Fat symmetry | Edema | Volume    | Fatty | Fat symmetry | Edema |
| upper girdle                  | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| biceps brachii                | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 1         | 3     | 1            | 1     |
| brachialis                    | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| triceps brachii               | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| forearm extensors             | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| forearm flexors               | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| thoracic wall                 | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| abdominal wall                | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| paraspinal cervical, thoracic | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 3     | 1            | 0     |
| paraspinal L/S                | 0         | 0     | 1            | 0     | 0         | 2     | 1            | 1     | 0         | 1     | 1            | 0     | 0         | 4     | 1            | 2     |
| gluteus maximus               | 0         | 0     | 1            | 0     | 1         | 2     | 1            | 0     | 1         | 1     | 1            | 0     | 0         | 2     | 1            | 0     |
| gluteus medius                | 0         | 0     | 1            | 0     | 0         | 2     | 0            | 2     | 0         | 0     | 1            | 0     | 0         | 3     | 1            | 2     |
| gluteus minimus               | 0         | 1     | 1            | 0     | 1         | 3     | 1            | 2     | 0         | 1     | 1            | 0     | 0         | 4     | 1            | 0     |
| sartorius                     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 1     | 1            | 0     | 0         | 1     | 1            | 0     |
| tensor fasciae latae          | 0         | 0     | 1            | 0     | 1         | 3     | 0            | 0     | 0         | 1     | 1            | 0     | 0         | 4     | 1            | 0     |
| adductor longus               | 1         | 3     | 0            | 1     | 0         | 4     | 0            | 2     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| adductor magnus               | 0         | 0     | 1            | 0     | 1         | 2     | 0            | 2     | 0         | 1     | 1            | 0     | 1         | 3     | 1            | 0     |
| gracilis                      | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| rectus femoris                | 0         | 0     | 1            | 0     | 0         | 1     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| vastus lateralis              | 1         | 1     | 0            | 1     | 0         | 2     | 0            | 1     | 0         | 0     | 1            | 0     | 1         | 1     | 1            | 0     |
| vastus medialis               | 0         | 0     | 1            | 1     | 0         | 3     | 0            | 1     | 0         | 0     | 1            | 0     | 1         | 3     | 0            | 1     |
| vastus intermedius            | 0         | 0     | 1            | 0     | 1         | 2     | 0            | 2     | 0         | 0     | 1            | 0     | 1         | 1     | 1            | 0     |
| biceps femoris long head      | 1         | 1     | 0            | 1     | 1         | 1     | 1            | 1     | 1         | 1     | 1            | 0     | 1         | 4     | 1            | 0     |
| biceps femoris short head     | 0         | 0     | 1            | 0     | 1         | 2     | 1            | 1     | 0         | 0     | 0            | 0     | 1         | 2     | 1            | 2     |
| semimembranosus               | 1         | 2     | 0            | 1     | 1         | 2     | 0            | 1     | 1         | 1     | 1            | 0     | 1         | 4     | 1            | 0     |
| semitendinosus                | 1         | 1     | 1            | 0     | 1         | 1     | 1            | 0     | 0         | 0     | 1            | 0     | 1         | 3     | 1            | 1     |
| tibialis anterior             | 0         | 0     | 1            | 0     | 0         | 2     | 0            | 1     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| peroneus                      | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| medial gastrocnemius          | 1         | 3     | 1            | 0     | 1         | 4     | 1            | 0     | 0         | 2     | 1            | 2     | 2         | 4     | 1            | 0     |
| lateral gastrocnemius         | 0         | 0     | 1            | 0     | 1         | 3     | 0            | 2     | 0         | 0     | 1            | 0     | 1         | 4     | 0            | 1     |
| soleus                        | 1         | 1     | 0            | 1     | 1         | 1     | 1            | 1     | 0         | 0     | 1            | 0     | 2         | 4     | 1            | 1     |
| tibialis posterior            | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     |
| flexor hallucis longus        | 1         | 1     | 0            | 2     | 0         | 0     | 1            | 0     | 0         | 0     | 1            | 0     | 2         | 3     | 0            | 2     |

Legend to the parameters:

Volume :

- 0=no change
- 1=muscle atrophy
- 2=hypertrophy

Fat:

Fatty degeneration according to modified  
Fischer grading scale:

- 0=no fatty degeneration
- 1=Mild
- 2=Moderate
- 3=Severe
- 4=Entire Muscle

Fat  
Symmetry:

- 0= asymetric fatty degeneration
- 1=symmetry

Edema:

- 0 = no edema
- 1= symmetrical  
edema
- 2=asymmetrical  
edema

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Supplementary Material 3

WES Analysis of Patients 3-5

## patient 3

**Proband ID:** MWAR023  
**Inferred Sex (Reported):** Male (Male)  
**Inferred Ancestry (Reported):** European (European)

### Candidate Mutations

| Gene<br>Transcript<br>Genotype <sup>4</sup>    | Gene Variant <sup>1</sup><br>Transcript Change <sup>5</sup><br>Protein Change<br>dbSNP <sup>7</sup> | ExAC AF <sup>2</sup><br>Highest Pop <sup>6</sup> | ClinVar <sup>3</sup><br>ClinVar Confidence<br>Stars | Notes |
|------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------------------------|-----------------------------------------------------|-------|
| <b>RYR1</b><br>ENST00000359596.3<br><i>het</i> | 19:38954139 G>A<br>c.2654G>A<br><b>p.Arg885His</b><br>rs370634440                                   | 1.77e-04<br>3.66e-04                             | NA                                                  |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i> | 11:22283708 G>T<br>c.1664G>T<br><b>p.Ser555Ile</b><br>rs375014127                                   | 7.42e-05<br>1.51e-04                             | NA                                                  |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i> | 11:22225378 C>A<br>c.69C>A<br><b>p.Tyr23Ter</b>                                                     | 9.82e-06<br>1.84e-05                             | NA                                                  |       |

### Footnotes:

<sup>1</sup> Genomic variants are based on build37/hg19. This is a filtered list of variants containing **nonsense**, **frameshift**, **essential splice** and **missense** variants.

<sup>2</sup> ExAC Allele Frequency (AF). For more information on ExAC please refer to *General Methodology* section.

<sup>3</sup> For more details on ClinVar and how confidence is rated please refer to *General Methodology* section.

<sup>4</sup> Genotype is either heterozygous (*het*), homozygous (*hom*) or hemizygous (*hem*) for the variant.

<sup>5</sup> Transcript changes are based on Ensembl.

<sup>6</sup> Highest allele frequency in any of the given ExAC populations (Pop).

<sup>7</sup> rsID if present in dbSNP version 141.

<sup>+</sup> Large insertion/deletions have been truncated.

### Limitations:

- Analysis limited to gene list. For more information on gene list please refer to *Appendix*.
- This exome was sequenced in a research environment and results have not been clinically validated. Clinical validation of candidate causal variants should be performed prior to making a formal clinical diagnosis. Please refer to *Appendix* for coverage of this sample.
- Proband only approach used in this project and thus unable to determine phasing of two or more variants (whether in *cis* or *trans*) and also unable to determine if mutations are inherited or de novo. Determining phasing and inheritance of variants requires sequencing of parents.

## Appendix - Candidate Gene List

### All Rare Variants Called

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------|
| <b>ABHD5</b><br>ENST00000458276.2<br><i>hom</i>           | 3:43756577 C>CTG<br>c.773+28..773+29[4]TG                                                                                | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127931 A>C<br>c.1748-30A>C                                                                                           | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127937 A>C<br>c.1748-24A>C                                                                                           | 1.18e-03<br>3.85e-03                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127943 A>C<br>c.1748-18A>C                                                                                           | 1.08e-03<br>7.52e-03                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127948 A>C<br>c.1748-13A>C                                                                                           | 5.60e-04<br>6.34e-03                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127951 T>C<br>c.1748-10T>C                                                                                           | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127952 A>C<br>c.1748-9A>C                                                                                            | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i>            | 11:22225378 C>A<br>c.69C>A<br><b>p.Tyr23Ter</b>                                                                          | 9.82e-06<br>1.84e-05                                    | NA                                                |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i>            | 11:22283708 G>T<br>c.1664G>T<br><b>p.Ser555Ile</b>                                                                       | 7.42e-05<br>1.51e-04                                    | NA                                                |       |

| Gene<br><i>Transcript<br/>Genotype<sup>4</sup></i> | Gene Variant <sup>1</sup><br><i>Transcript Change<sup>5</sup><br/>Protein Change<br/>dbSNP<sup>7</sup></i> | ExAC AF <sup>2</sup><br><i>Highest Pop<sup>6</sup></i> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|----------------------------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------|-------|
|                                                    | rs375014127                                                                                                |                                                        |                                                   |       |
| <b>CHKB</b><br>ENST00000406938.2<br><i>het</i>     | 22:51021258 C>A<br>c.-48G>T                                                                                | 5.09e-03<br>1.69e-02                                   | NA                                                |       |
| <b>DMD</b><br>ENST00000357033.4<br><i>het</i>      | X:31950370 A>AT<br>c.6615-27delA                                                                           | 7.89e-04<br>3.07e-03                                   | NA                                                |       |
| <b>DYSF</b><br>ENST00000410020.3<br><i>het</i>     | 2:71825707 C>T<br>c.3588C>T<br>c.3588C>T(p.=)                                                              | 7.36e-03<br>1.16e-02                                   | NA                                                |       |
| <b>FAM111B</b><br>ENST00000343597.3<br><i>het</i>  | 11:58892376 C>CA<br>c.807delA<br>p.Ala273HisfsTer26                                                        | 4.04e-03<br>6.72e-03                                   | NA                                                |       |
| <b>GBE1</b><br>ENST00000429644.2<br><i>het</i>     | 3:81719954 A>G<br>c.429+35T>C                                                                              | 5.34e-03<br>9.06e-03                                   | NA                                                |       |
| <b>GMPPB</b><br>ENST00000308375.6<br><i>het</i>    | 3:49760503 C>G<br>c.304G>C<br>p.Asp102His<br>rs368542417                                                   | 4.14e-05<br>7.54e-05                                   | NA                                                |       |
| <b>MUSK</b><br>ENST00000374448.4<br><i>het</i>     | 9:113562589 T>C<br>c.1931T>C<br>p.Val644Ala<br>rs41279055                                                  | 2.84e-03<br>4.22e-03                                   | NA                                                |       |
| <b>MYBPC3</b><br>ENST00000545968.1<br><i>het</i>   | 11:47368208 T>G<br>c.909-13A>C                                                                             | 5.78e-05<br>3.29e-04                                   | NA                                                |       |
| <b>MYH2</b><br>ENST00000245503.5<br><i>het</i>     | 17:10428348 C>T<br>c.4697G>A<br>p.Arg1566His<br>rs200732220                                                | 1.73e-04<br>3.00e-04                                   | NA                                                |       |
| <b>MYH3</b><br>ENST00000583535.1                   | 17:10555926 CTTT>C<br>c.205-62_205-47delAAAAAAAAAAAAA*                                                     | 0.00e+00<br>0.00e+00                                   | NA                                                |       |

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------|
| <i>het</i>                                                |                                                                                                                          |                                                         |                                                   |       |
| <b>MYH3</b><br>ENST00000583535.1<br><i>het</i>            | 17:10555926 CTTT>C<br>c.205-62_205-47delAAAAAAAAAAAAA <sup>+</sup>                                                       | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>NEB</b><br>ENST00000397345.3<br><i>het</i>             | 2:152544120 G>C<br>c.2523+20C>G                                                                                          | 2.53e-03<br>2.26e-02                                    | NA                                                |       |
| <b>OPA1</b><br>ENST00000361908.3<br><i>het</i>            | 3:193332466 A>C<br>c.33-46A>C                                                                                            | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>PLEC</b><br>ENST00000322810.4<br><i>het</i>            | 8:144992269 G>A<br>c.12131C>T<br>p.Thr4044Met<br>rs78461695                                                              | 9.26e-03<br>1.30e-02                                    | NA                                                |       |
| <b>PLEC</b><br>ENST00000322810.4<br><i>het</i>            | 8:144999731 C>T<br>c.4777G>A<br>p.Val1593Met<br>rs186848953                                                              | 4.79e-03<br>7.23e-03                                    | NA                                                |       |
| <b>PLEC</b><br>ENST00000322810.4<br><i>het</i>            | 8:145011204 A>C<br>c.789T>G<br>c.789T>G(p.=)                                                                             | 2.79e-03<br>4.81e-03                                    | NA                                                |       |
| <b>RYR1</b><br>ENST00000359596.3<br><i>het</i>            | 19:38954139 G>A<br>c.2654G>A<br>p.Arg885His<br>rs370634440                                                               | 1.77e-04<br>3.66e-04                                    | NA                                                |       |
| <b>RYR1</b><br>ENST00000359596.3<br><i>het</i>            | 19:39062962 T>G<br>c.13998+52delTinsTGGGGGGGGGGGGG <sup>+</sup>                                                          | 1.47e-03<br>2.81e-03                                    | NA                                                |       |
| <b>SCN4A</b><br>ENST00000435607.1<br><i>het</i>           | 17:62038535 T>C<br>c.1845+17_1845+18delGAinsGGA                                                                          | 2.39e-03<br>4.07e-03                                    | NA                                                |       |
| <b>SGCA</b>                                               | 17:48246702 G>A                                                                                                          | 7.56e-03                                                | NA                                                |       |

| Gene<br>Transcript<br>Genotype <sup>4</sup>      | Gene Variant <sup>1</sup><br>Transcript Change <sup>5</sup><br>Protein Change<br>dbSNP <sup>7</sup> | ExAC AF <sup>2</sup><br>Highest Pop <sup>6</sup> | ClinVar <sup>3</sup><br>ClinVar Confidence | Notes |
|--------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------------------------|--------------------------------------------|-------|
| ENST00000451235.2<br><i>het</i>                  | c.528G>A<br>c.528G>A(p.=)                                                                           | 5.46e-02                                         |                                            |       |
| <b>STIM2</b><br>ENST00000465503.1<br><i>het</i>  | 4:27004737 T>TA<br>c.981+11_981+12delTAinsGA                                                        | 4.87e-03<br>1.19e-02                             | NA                                         |       |
| <b>NUDT15</b><br>ENST00000258662.2<br><i>het</i> | 13:48611918 A>C<br>c.36_42delIAGGAGTCinsAGGAGTCGGA <sup>+</sup><br>p.Val14_Gly15insGlyVal           | 3.43e-03<br>6.45e-03                             | NA                                         |       |
| <b>SYNE1</b><br>ENST00000367255.5<br><i>het</i>  | 6:152690074 TCA>T<br>c.9807+31_9807+32delTG                                                         | 0.00e+00<br>0.00e+00                             | NA                                         |       |
| <b>SYNE1</b><br>ENST00000367255.5<br><i>het</i>  | 6:152712715 G>GAAAAAAAAA<br>c.7713-13delT                                                           | 6.49e-03<br>1.26e-02                             | NA                                         |       |
| <b>SYNE1</b><br>ENST00000367255.5<br><i>het</i>  | 6:152737573 C>T<br>c.5999G>A<br>p.Arg2000Lys<br>rs149146258                                         | 1.58e-03<br>5.59e-03                             | NA                                         |       |
| <b>TK2</b><br>ENST00000451102.2<br><i>het</i>    | 16:66575895 G>A<br>c.157-39C>T                                                                      | 2.29e-03<br>3.81e-03                             | NA                                         |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>    | 2:179553730 AT>A<br>c.32095+42_32095+49delAAAAAAAAA                                                 | 6.92e-04<br>2.29e-03                             | NA                                         |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>    | 2:179582769 C>A<br>c.24964G>T<br>p.Val8322Leu<br>rs201571580                                        | 7.54e-04<br>1.33e-03                             | NA                                         |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>    | 2:179584211 A>G<br>c.23939-33T>C                                                                    | 0.00e+00<br>0.00e+00                             | NA                                         |       |

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------|
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>             | 2:179596554 T>C<br>c.17048A>G<br><b>p.Tyr5683Cys</b><br>rs72648942                                                       | 5.85e-03<br>8.56e-03                                    | NA                                                |       |
| <b>VAPB</b><br>ENST00000475243.1<br><i>het</i>            | 20:56964367 CAGCG>C<br>c.-149_-143delCAGCGCGinsC                                                                         | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>VAPB</b><br>ENST00000475243.1<br><i>het</i>            | 20:56964373 G>C<br>c.-143G>C                                                                                             | 0.00e+00<br>0.00e+00                                    | NA                                                |       |

**Footnotes:**

<sup>1</sup>Genomic variants are based on build37/hg19. This is a filtered list (AF <0.01) of variants containing **non-sense, frameshift, essential splice, missense** and **synonymous** variants.

<sup>2</sup>ExAC Allele Frequency (AF). For more information on ExAC please refer to *General Methodology* section.

<sup>3</sup>For more details on ClinVar and how confidence is rated please refer to *General Methodology* section.

<sup>4</sup>Genotype is either heterozygous (*het*), homozygous (*hom*) or hemizygous (*hem*) for the variant.

<sup>5</sup>Transcript changes are based on Ensembl.

<sup>6</sup>Highest allele frequency in any of the given ExAC populations (Pop).

<sup>7</sup>rsID if present in dbSNP version 141.

<sup>+</sup>Large insertion/deletions have been truncated.

## Gene Coverage

[illegible]

| Gene    | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|---------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|         | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| COLQ    | 90.89                 | 93.15               | 100.00                          | 100.00 | 0                             | 0      |
| COX15   | 95.34                 | 95.59               | 100.00                          | 100.00 | 0                             | 0      |
| CPT2    | 87.59                 | 85.07               | 100.00                          | 99.28  | 0                             | 15     |
| CRYAB   | 87.02                 | 88.57               | 100.00                          | 100.00 | 0                             | 0      |
| DAG1    | 94.02                 | 92.98               | 100.00                          | 100.00 | 0                             | 0      |
| DES     | 82.38                 | 77.44               | 100.00                          | 95.28  | 0                             | 75     |
| DMD     | 61.42                 | 49.05               | 100.00                          | 99.69  | 0                             | 40     |
| DNAJB6  | 41.09                 | 34.03               | 100.00                          | 100.00 | 0                             | 0      |
| DNM2    | 86.07                 | 85.49               | 97.80                           | 96.43  | 82                            | 133    |
| DOK7    | 72.30                 | 66.74               | 100.00                          | 95.52  | 0                             | 74     |
| DOLK    | 93.48                 | 92.52               | 100.00                          | 100.00 | 0                             | 0      |
| DPAGT1  | 93.24                 | 93.77               | 100.00                          | 100.00 | 0                             | 0      |
| DPM1    | 62.80                 | 67.45               | 93.18                           | 89.77  | 74                            | 111    |
| DPM2    | 27.88                 | 25.52               | 100.00                          | 100.00 | 0                             | 0      |
| DPM3    | 84.43                 | 86.07               | 100.00                          | 100.00 | 0                             | 0      |
| DYSF    | 92.97                 | 93.45               | 100.00                          | 100.00 | 0                             | 0      |
| EMD     | 69.98                 | 56.27               | 100.00                          | 100.00 | 0                             | 0      |
| ENO3    | 97.90                 | 97.40               | 100.00                          | 100.00 | 0                             | 0      |
| ETFA    | 53.56                 | 56.59               | 86.15                           | 87.07  | 256                           | 239    |
| ETFB    | 98.62                 | 98.06               | 100.00                          | 100.00 | 0                             | 0      |
| ETFDH   | 58.38                 | 57.87               | 100.00                          | 100.00 | 0                             | 0      |
| FAM111B | 53.15                 | 53.90               | 99.42                           | 99.11  | 13                            | 20     |
| FHL1    | 69.09                 | 56.54               | 100.00                          | 100.00 | 0                             | 0      |
| FKBP14  | 24.56                 | 25.57               | 100.00                          | 99.59  | 0                             | 3      |
| FKRP    | 55.29                 | 45.65               | 100.00                          | 79.27  | 0                             | 312    |
| FKTN    | 66.04                 | 70.62               | 97.07                           | 97.07  | 56                            | 56     |
| FLNC    | 94.92                 | 94.24               | 100.00                          | 100.00 | 0                             | 0      |
| GAA     | 84.62                 | 84.02               | 100.00                          | 100.00 | 0                             | 0      |
| GBE1    | 27.25                 | 27.40               | 100.00                          | 100.00 | 0                             | 0      |
| GFPT1   | 73.05                 | 76.11               | 100.00                          | 100.00 | 0                             | 0      |
| GMPPB   | 63.96                 | 54.27               | 100.00                          | 100.00 | 0                             | 0      |
| GNE     | 83.70                 | 85.63               | 100.00                          | 100.00 | 0                             | 0      |
| GYG1    | 77.76                 | 77.41               | 100.00                          | 100.00 | 0                             | 0      |
| GYS1    | 88.92                 | 88.40               | 100.00                          | 100.00 | 0                             | 0      |
| HNRNPDL | 39.25                 | 37.67               | 90.00                           | 87.14  | 140                           | 180    |
| IGHMBP2 | 90.87                 | 92.15               | 100.00                          | 100.00 | 0                             | 0      |

| Gene    | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|---------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|         | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| ISCU    | 69.30                 | 69.53               | 100.00                          | 93.99  | 0                             | 53     |
| ISPD    | 32.81                 | 31.05               | 82.16                           | 82.03  | 277                           | 279    |
| ITGA7   | 90.12                 | 90.11               | 100.00                          | 100.00 | 0                             | 0      |
| KBTBD13 | 49.11                 | 42.71               | 96.77                           | 84.07  | 45                            | 222    |
| KCNJ2   | 99.05                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| KLHL40  | 71.73                 | 67.16               | 100.00                          | 100.00 | 0                             | 0      |
| KLHL41  | 53.63                 | 53.14               | 100.00                          | 100.00 | 0                             | 0      |
| KLHL9   | 80.33                 | 79.18               | 100.00                          | 100.00 | 0                             | 0      |
| LAMA2   | 85.80                 | 88.22               | 100.00                          | 100.00 | 0                             | 0      |
| LAMB2   | 97.05                 | 96.86               | 100.00                          | 100.00 | 0                             | 0      |
| LAMP2   | 50.06                 | 38.73               | 100.00                          | 97.45  | 0                             | 44     |
| LARGE   | 90.15                 | 90.99               | 100.00                          | 100.00 | 0                             | 0      |
| LDB3    | 89.95                 | 89.63               | 100.00                          | 100.00 | 0                             | 0      |
| LDHA    | 70.61                 | 69.39               | 100.00                          | 100.00 | 0                             | 0      |
| LIMS2   | 55.94                 | 54.38               | 90.90                           | 90.90  | 134                           | 134    |
| LMNA    | 88.12                 | 86.87               | 100.00                          | 100.00 | 0                             | 0      |
| LPIN1   | 76.22                 | 76.58               | 100.00                          | 100.00 | 0                             | 0      |
| LRP4    | 95.67                 | 95.83               | 100.00                          | 100.00 | 0                             | 0      |
| MATR3   | 67.55                 | 68.16               | 96.08                           | 95.54  | 123                           | 140    |
| MEGF10  | 46.71                 | 47.98               | 100.00                          | 99.95  | 0                             | 2      |
| MSTN    | 72.08                 | 73.08               | 100.00                          | 100.00 | 0                             | 0      |
| MTM1    | 54.57                 | 43.72               | 100.00                          | 100.00 | 0                             | 0      |
| MTMR14  | 85.21                 | 83.43               | 100.00                          | 99.81  | 0                             | 5      |
| MTTP    | 80.04                 | 82.94               | 100.00                          | 100.00 | 0                             | 0      |
| MUSK    | 52.22                 | 53.35               | 100.00                          | 99.51  | 0                             | 15     |
| MYBPC3  | 50.86                 | 48.70               | 99.47                           | 99.31  | 24                            | 31     |
| MYF6    | 90.16                 | 91.40               | 100.00                          | 100.00 | 0                             | 0      |
| MYH14   | 68.18                 | 67.30               | 100.00                          | 99.05  | 0                             | 66     |
| MYH2    | 91.34                 | 93.88               | 100.00                          | 100.00 | 0                             | 0      |
| MYH3    | 95.96                 | 97.18               | 100.00                          | 100.00 | 0                             | 0      |
| MYH7    | 97.49                 | 97.92               | 100.00                          | 100.00 | 0                             | 0      |
| MYOT    | 75.98                 | 78.45               | 100.00                          | 100.00 | 0                             | 0      |
| NEB     | 45.79                 | 46.59               | 87.75                           | 86.29  | 3589                          | 4018   |
| OPA1    | 62.12                 | 65.98               | 100.00                          | 100.00 | 0                             | 0      |
| ORAI1   | 83.27                 | 84.74               | 89.78                           | 88.30  | 97                            | 111    |
| PABPN1  | 68.54                 | 63.35               | 92.39                           | 83.38  | 81                            | 177    |

| Gene     | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|----------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|          | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| PFKM     | 85.41                 | 85.32               | 100.00                          | 99.97  | 0                             | 1      |
| PGAM2    | 99.17                 | 99.06               | 100.00                          | 100.00 | 0                             | 0      |
| PGK1     | 77.92                 | 59.77               | 100.00                          | 100.00 | 0                             | 0      |
| PGM1     | 91.24                 | 94.24               | 100.00                          | 100.00 | 0                             | 0      |
| PHKA1    | 66.18                 | 51.71               | 100.00                          | 100.00 | 0                             | 0      |
| PLEC     | 85.21                 | 76.54               | 99.77                           | 99.20  | 36                            | 127    |
| PNPLA2   | 78.73                 | 69.18               | 99.59                           | 98.88  | 7                             | 19     |
| POLG     | 91.70                 | 89.56               | 99.00                           | 96.69  | 42                            | 139    |
| POLG2    | 67.30                 | 68.15               | 100.00                          | 100.00 | 0                             | 0      |
| POMGNT1  | 90.97                 | 91.36               | 100.00                          | 100.00 | 0                             | 0      |
| POMGNT2  | 82.97                 | 80.52               | 100.00                          | 100.00 | 0                             | 0      |
| POMK     | 81.71                 | 81.72               | 100.00                          | 100.00 | 0                             | 0      |
| POMT1    | 93.06                 | 93.05               | 100.00                          | 100.00 | 0                             | 0      |
| POMT2    | 90.34                 | 91.14               | 100.00                          | 99.97  | 0                             | 1      |
| PREPL    | 45.05                 | 45.88               | 99.96                           | 100.00 | 1                             | 0      |
| PRKAG2   | 75.44                 | 76.52               | 100.00                          | 100.00 | 0                             | 0      |
| PTPLA    | 22.56                 | 21.57               | 97.86                           | 91.27  | 27                            | 110    |
| PTRF     | 84.71                 | 82.15               | 99.34                           | 96.12  | 8                             | 47     |
| PYGM     | 98.79                 | 98.76               | 100.00                          | 100.00 | 0                             | 0      |
| RAPSN    | 84.64                 | 84.62               | 100.00                          | 100.00 | 0                             | 0      |
| RRM2B    | 68.60                 | 72.14               | 91.25                           | 91.25  | 141                           | 141    |
| RYR1     | 91.80                 | 91.47               | 97.64                           | 96.58  | 412                           | 598    |
| SCN4A    | 74.92                 | 74.53               | 100.00                          | 100.00 | 0                             | 0      |
| SEPN1    | 80.43                 | 80.45               | 89.90                           | 89.90  | 205                           | 205    |
| SGCA     | 88.28                 | 87.37               | 100.00                          | 100.00 | 0                             | 0      |
| SGCB     | 68.02                 | 74.20               | 99.40                           | 95.44  | 7                             | 53     |
| SGCD     | 37.72                 | 37.98               | 100.00                          | 100.00 | 0                             | 0      |
| SGCG     | 86.15                 | 91.83               | 100.00                          | 100.00 | 0                             | 0      |
| SIL1     | 94.85                 | 96.40               | 100.00                          | 100.00 | 0                             | 0      |
| SLC22A5  | 92.52                 | 90.99               | 100.00                          | 100.00 | 0                             | 0      |
| SLC25A20 | 93.29                 | 93.48               | 100.00                          | 100.00 | 0                             | 0      |
| SLC25A4  | 76.10                 | 78.28               | 100.00                          | 100.00 | 0                             | 0      |
| SLC52A3  | 93.42                 | 93.00               | 100.00                          | 100.00 | 0                             | 0      |
| SMCHD1   | 24.46                 | 25.14               | 98.56                           | 96.24  | 102                           | 266    |
| STAC3    | 60.18                 | 56.46               | 100.00                          | 99.85  | 0                             | 2      |
| STIM1    | 86.56                 | 85.92               | 100.00                          | 100.00 | 0                             | 0      |

| Gene     | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|----------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|          | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| STIM2    | 43.79                 | 43.42               | 98.34                           | 96.54  | 47                            | 98     |
| SUCLA2   | 70.70                 | 76.50               | 100.00                          | 100.00 | 0                             | 0      |
| SYNE1    | 84.25                 | 86.32               | 100.00                          | 100.00 | 0                             | 0      |
| SYNE2    | 74.71                 | 77.40               | 99.98                           | 99.99  | 4                             | 3      |
| TARDBP   | 67.48                 | 66.98               | 89.56                           | 89.56  | 165                           | 165    |
| TAZ      | 81.92                 | 69.44               | 100.00                          | 100.00 | 0                             | 0      |
| TCAP     | 83.34                 | 84.49               | 100.00                          | 100.00 | 0                             | 0      |
| TIA1     | 32.56                 | 34.64               | 100.00                          | 100.00 | 0                             | 0      |
| TK2      | 77.13                 | 76.14               | 94.97                           | 92.17  | 68                            | 106    |
| TMEM43   | 74.28                 | 73.08               | 100.00                          | 100.00 | 0                             | 0      |
| TMEM5    | 31.07                 | 32.61               | 100.00                          | 96.81  | 0                             | 48     |
| TNNI2    | 93.91                 | 91.76               | 100.00                          | 100.00 | 0                             | 0      |
| TNNT1    | 69.98                 | 71.63               | 95.35                           | 89.02  | 64                            | 151    |
| TNNT3    | 99.16                 | 99.23               | 100.00                          | 100.00 | 0                             | 0      |
| TNPO3    | 44.98                 | 45.42               | 100.00                          | 99.82  | 0                             | 6      |
| TOR1AIP1 | 51.16                 | 49.69               | 97.51                           | 95.44  | 55                            | 101    |
| TPM2     | 90.27                 | 89.60               | 100.00                          | 100.00 | 0                             | 0      |
| TPM3     | 96.87                 | 98.21               | 100.00                          | 100.00 | 0                             | 0      |
| TRAPPC11 | 37.43                 | 37.19               | 100.00                          | 100.00 | 0                             | 0      |
| TRIM32   | 94.96                 | 96.18               | 100.00                          | 100.00 | 0                             | 0      |
| TTN      | 58.05                 | 56.11               | 98.35                           | 97.48  | 2002                          | 3061   |
| UBA1     | 85.50                 | 76.31               | 99.98                           | 98.23  | 1                             | 73     |
| VAPB     | 80.46                 | 82.12               | 100.00                          | 100.00 | 0                             | 0      |
| VCP      | 98.26                 | 98.18               | 100.00                          | 100.00 | 0                             | 0      |
| VMA21    | 41.32                 | 33.88               | 100.00                          | 100.00 | 0                             | 0      |
| YARS2    | 83.53                 | 87.22               | 100.00                          | 100.00 | 0                             | 0      |

**Notes:**

<sup>1</sup>Average per-base coverage for gene. Genes that are not completely callable are highlighted in pink. For more information on how coverage was calculated, please refer to *General Methodology* section.

<sup>2</sup>The percent of sites that are considered callable for this gene. For more information on how callability is determined, please refer to *General Methodology* section.

<sup>3</sup>The average for samples included in the cohort.

<sup>4</sup>The metric for the sample in this report.

**patient 4**

**Proband ID:** MWAR022  
**Inferred Sex (Reported):** Female (Female)  
**Inferred Ancestry (Reported):** European (European)

### Candidate Mutations

| Gene<br>Transcript<br>Genotype <sup>4</sup>    | Gene Variant <sup>1</sup><br>Transcript Change <sup>5</sup><br>Protein Change<br>dbSNP <sup>7</sup> | ExAC AF <sup>2</sup><br>Highest Pop <sup>6</sup> | ClinVar <sup>3</sup><br>ClinVar Confidence<br>Stars | Notes |
|------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------------------------|-----------------------------------------------------|-------|
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i> | 11:22248879 A>T<br>c.395A>T<br><b>p.Lys132Met</b>                                                   | 0.00e+00<br>0.00e+00                             | NA                                                  |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i> | 11:22301090 C>G<br>c.2521C>G<br><b>p.His841Asp</b>                                                  | 1.67e-05<br>3.04e-05                             | NA                                                  |       |

### Footnotes:

<sup>1</sup>Genomic variants are based on build37/hg19. This is a filtered list of variants containing **nonsense, frameshift, essential splice** and **missense** variants.

<sup>2</sup>ExAC Allele Frequency (AF). For more information on ExAC please refer to *General Methodology* section.

<sup>3</sup>For more details on ClinVar and how confidence is rated please refer to *General Methodology* section.

<sup>4</sup>Genotype is either heterozygous (*het*), homozygous (*hom*) or hemizygous (*hem*) for the variant.

<sup>5</sup>Transcript changes are based on Ensembl.

<sup>6</sup>Highest allele frequency in any of the given ExAC populations (Pop).

<sup>7</sup>rsID if present in dbSNP version 141.

<sup>+</sup>Large insertion/deletions have been truncated.

### Limitations:

- Analysis limited to gene list. For more information on gene list please refer to *Appendix*.
- This exome was sequenced in a research environment and results have not been clinically validated. Clinical validation of candidate causal variants should be performed prior to making a formal clinical diagnosis. Please refer to *Appendix* for coverage of this sample.
- Proband only approach used in this project and thus unable to determine phasing of two or more variants (whether in *cis* or *trans*) and also unable to determine if mutations are inherited or de novo. Determining phasing and inheritance of variants requires sequencing of parents.

## Appendix - Candidate Gene List

### All Rare Variants Called

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------|
| <b>ABHD5</b><br>ENST00000458276.2<br><i>het</i>           | 3:43756577 C>CTG<br>c.773+28_773+29[4]TG                                                                                 | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>ABHD5</b><br>ENST00000458276.2<br><i>het</i>           | 3:43756579 G>C<br>c.773+29G>C                                                                                            | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127931 A>ACCCCCC<br>c.1748-30delAinsACCCCCC                                                                          | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127937 A>C<br>c.1748-24A>C                                                                                           | 1.18e-03<br>3.85e-03                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127943 A>C<br>c.1748-18A>C                                                                                           | 1.08e-03<br>7.52e-03                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127948 A>C<br>c.1748-13A>C                                                                                           | 5.60e-04<br>6.34e-03                                    | NA                                                |       |
| <b>AGRN</b><br>ENST00000379370.2<br><i>het</i>            | 1:981340 C>T<br>c.2681-4C>T                                                                                              | 8.11e-04<br>1.30e-03                                    | NA                                                |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i>            | 11:22248879 A>T<br>c.395A>T<br>p.Lys132Met                                                                               | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i>            | 11:22301090 C>G<br>c.2521C>G<br>p.His841Asp                                                                              | 1.67e-05<br>3.04e-05                                    | NA                                                |       |

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------|
|                                                           |                                                                                                                          |                                                         |                                                   |       |
| <b>AR</b><br>ENST00000374690.3<br><i>het</i>              | X:66765158 TGCA>T<br>c.171_182delGCAGCAGCAGCAinsGCA <sup>+</sup><br><b>p.Gln61dup</b>                                    | 3.15e-03<br>6.12e-03                                    | NA                                                |       |
| <b>AR</b><br>ENST00000374690.3<br><i>het</i>              | X:66765161 A>T<br>c.173A>T<br><b>p.Gln58Leu</b><br>rs200185441                                                           | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>BAG3</b><br>ENST00000369085.3<br><i>het</i>            | 10:121431904 C>T<br>c.645C>T<br><b>c.645C&gt;T(p.=)</b>                                                                  | 1.74e-04<br>4.54e-04                                    | NA                                                |       |
| <b>C10orf2</b><br>ENST00000311916.2<br><i>het</i>         | 10:102753257 G>A<br>c.2045G>A<br><b>p.Arg682His</b><br>rs182559752                                                       | 6.46e-04<br>2.97e-03                                    | NA                                                |       |
| <b>COL12A1</b><br>ENST00000322507.8<br><i>het</i>         | 6:75833783 C>T<br>c.6752G>A<br><b>p.Arg2251His</b><br>rs151324784                                                        | 3.73e-04<br>5.86e-04                                    | NA                                                |       |
| <b>COL12A1</b><br>ENST00000322507.8<br><i>het</i>         | 6:75890913 T>C<br>c.1906A>G<br><b>p.Lys636Glu</b>                                                                        | 8.37e-06<br>1.05e-04                                    | NA                                                |       |
| <b>DYSF</b><br>ENST00000410020.3<br><i>het</i>            | 2:71740897 C>A<br>c.605C>A<br><b>p.Ala202Glu</b><br>rs34999029                                                           | 9.81e-03<br>1.48e-02                                    | NA                                                |       |
| <b>DYSF</b><br>ENST00000410020.3<br><i>het</i>            | 2:71762387 T>C<br>c.1450-11T>C                                                                                           | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>FLNC</b><br>ENST00000325888.8<br><i>het</i>            | 7:128485240 C>T<br>c.3721C>T<br><b>p.Arg1241Cys</b><br>rs146953558                                                       | 6.29e-03<br>1.08e-02                                    | NA                                                |       |
| <b>LAMA2</b><br>ENST00000421865.2                         | 6:129601217 C>T<br>c.2462C>T                                                                                             | 2.03e-03<br>3.64e-03                                    | NA                                                |       |

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------|
| <i>het</i>                                                | p.Thr821Met<br>rs117422805                                                                                               |                                                         |                                                   |       |
| <b>MUSK</b><br>ENST00000374439.1<br><i>het</i>            | 9:113468417 C>T<br>c.336C>T<br>c.336C>T(p.=)                                                                             | 2.69e-03<br>4.64e-03                                    | NA                                                |       |
| <b>MYH14</b><br>ENST00000601313.1<br><i>het</i>           | 19:50770307 T>G<br>c.2826+42T>G                                                                                          | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>MYH3</b><br>ENST00000583535.1<br><i>het</i>            | 17:10555926 CTT>C<br>c.205-62_205-47delAAAAAAAAAAAAA <sup>+</sup>                                                        | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>MYH3</b><br>ENST00000583535.1<br><i>het</i>            | 17:10555926 CTTTTTTTTT <sup>+</sup> >C<br>c.205-63_205-47delAAAAAAAAAAAAA <sup>+</sup>                                   | 3.25e-03<br>7.84e-03                                    | NA                                                |       |
| <b>MYH3</b><br>ENST00000583535.1<br><i>het</i>            | 17:10555927 T>C<br>c.205-47A>G                                                                                           | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>MYH3</b><br>ENST00000583535.1<br><i>het</i>            | 17:10555929 T>C<br>c.205-49A>G                                                                                           | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>NEB</b><br>ENST00000397345.3<br><i>het</i>             | 2:152359276 ATT>A<br>c.23928+28_23928+30delAAA                                                                           | 6.06e-03<br>9.90e-03                                    | NA                                                |       |
| <b>NEB</b><br>ENST00000397345.3<br><i>het</i>             | 2:152370963 A>G<br>c.23017-21T>C                                                                                         | 6.20e-03<br>1.41e-02                                    | NA                                                |       |
| <b>NEB</b><br>ENST00000397345.3<br><i>het</i>             | 2:152410513 C>T<br>c.19455G>A<br>c.19455G>A(p.=)                                                                         | 3.39e-03<br>1.33e-02                                    | NA                                                |       |
| <b>PHKA1</b>                                              | X:71813193 T>TC                                                                                                          | 0.00e+00                                                | NA                                                |       |

| Gene<br><i>Transcript<br/>Genotype<sup>4</sup></i> | Gene Variant <sup>1</sup><br><i>Transcript Change<sup>5</sup><br/>Protein Change<br/>dbSNP<sup>7</sup></i> | ExAC AF <sup>2</sup><br><i>Highest Pop<sup>6</sup></i> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|----------------------------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------|-------|
| ENST00000373542.4<br><i>het</i>                    | c.3073-69delAinsGA                                                                                         | 0.00e+00                                               |                                                   |       |
| <b>PLEC</b><br>ENST00000322810.4<br><i>het</i>     | 8:144997370 G>T<br>c.7138C>A<br><b>p.Leu2380Ile</b><br>rs372627763                                         | 1.29e-04<br>2.35e-04                                   | NA                                                |       |
| <b>PLEC</b><br>ENST00000322810.4<br><i>het</i>     | 8:145011889 G>C<br>c.675+430C>G                                                                            | 1.87e-04<br>7.23e-04                                   | NA                                                |       |
| <b>PREPL</b><br>ENST00000409936.1<br><i>het</i>    | 2:44559583 G>A<br>c.1353+15C>T                                                                             | 6.82e-03<br>1.03e-02                                   | NA                                                |       |
| <b>RYR1</b><br>ENST00000359596.3<br><i>het</i>     | 19:38968521 T>G<br>c.4454+11T>G                                                                            | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>RYR1</b><br>ENST00000359596.3<br><i>het</i>     | 19:38993391 TGGGGCAGGG <sup>+</sup> >T<br>c.7835+25_7835+42delGGGGCAGGGG <sup>+</sup>                      | 2.53e-04<br>2.07e-03                                   | NA                                                |       |
| <b>RYR1</b><br>ENST00000359596.3<br><i>het</i>     | 19:39056020 GGGC>G<br>c.13047_13049delGGC<br><b>p.Ala4352del</b>                                           | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>SYNE1</b><br>ENST00000367255.5<br><i>het</i>    | 6:152712715 G>GA<br>c.7713-13delT                                                                          | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>SYNE2</b><br>ENST00000358025.3<br><i>het</i>    | 14:64443259 T>C<br>c.1129-22N>C                                                                            | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>SYNE2</b><br>ENST00000358025.3<br><i>het</i>    | 14:64453292 T>C<br>c.2270T>C<br><b>p.Leu757Ser</b><br>rs200319405                                          | 4.14e-04<br>7.04e-04                                   | NA                                                |       |

| Gene<br><i>Transcript<br/>Genotype<sup>4</sup></i> | Gene Variant <sup>1</sup><br><i>Transcript Change<sup>5</sup><br/>Protein Change<br/>dbSNP<sup>7</sup></i> | ExAC AF <sup>2</sup><br><i>Highest Pop<sup>6</sup></i> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|----------------------------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------|-------|
| <b>SYNE2</b><br>ENST00000358025.3<br><i>het</i>    | 14:64494308 C>G<br>c.6511C>G<br><b>p.Leu2171Val</b><br>rs199743242                                         | 4.23e-04<br>7.20e-04                                   | NA                                                |       |
| <b>TNNT1</b><br>ENST00000588981.1<br><i>hom</i>    | 19:55646893 C>CT<br>c.612-1325.612-1322delAAAA                                                             | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179478597 C>A<br>c.49413G>T<br><b>p.Trp16471Cys</b><br>rs202094100                                       | 3.92e-04<br>6.32e-04                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179478746 C>CT<br>c.49345+32delA                                                                         | 7.50e-03<br>2.99e-02                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179519303 T>C<br>c.38207-42A>G                                                                           | 3.33e-03<br>1.94e-02                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179544076 C>T<br>c.33732G>A<br><b>c.33732G&gt;A(p.=)</b>                                                 | 2.58e-04<br>4.24e-04                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179553730 ATT>A<br>c.32095+42_32095+49delAAAAAAAAA                                                       | 1.41e-03<br>3.44e-03                                   | NA                                                |       |
| <b>TTN</b><br><i>het</i>                           | 2:179553755 G>T                                                                                            | 5.93e-04<br>7.97e-04                                   | NA                                                |       |
| <b>UBA1</b><br>ENST00000335972.6<br><i>het</i>     | X:47063147 T>TA<br>c.1575+52delTinsTA                                                                      | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>YARS2</b><br>ENST00000324868.8<br><i>het</i>    | 12:32905700 G>A<br>c.947+1152C>T                                                                           | 9.33e-04<br>1.30e-03                                   | NA                                                |       |

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------|
|                                                           |                                                                                                                          |                                                         |                                                   |       |
| <b>YARS2</b><br>ENST00000324868.8<br><i>het</i>           | 12:32905701 ATTATC>A<br>c.947+1146_947+1150delGATAA                                                                      | 3.20e-04<br>4.54e-04                                    | NA                                                |       |

**Footnotes:**

<sup>1</sup>Genomic variants are based on build37/hg19. This is a filtered list (AF <0.01) of variants containing **non-sense, frameshift, essential splice, missense** and **synonymous** variants.

<sup>2</sup>ExAC Allele Frequency (AF). For more information on ExAC please refer to *General Methodology* section.

<sup>3</sup>For more details on ClinVar and how confidence is rated please refer to *General Methodology* section.

<sup>4</sup>Genotype is either heterozygous (*het*), homozygous (*hom*) or hemizygous (*hem*) for the variant.

<sup>5</sup>Transcript changes are based on Ensembl.

<sup>6</sup>Highest allele frequency in any of the given ExAC populations (Pop).

<sup>7</sup>rsID if present in dbSNP version 141.

<sup>+</sup>Large insertion/deletions have been truncated.

## Gene Coverage

| Gene     | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|----------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|          | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| ABHD5    | 81.52                 | 78.25               | 100.00                          | 100.00 | 0                             | 0      |
| ACADS    | 93.78                 | 96.85               | 100.00                          | 100.00 | 0                             | 0      |
| ACADVL   | 92.82                 | 95.61               | 98.24                           | 98.24  | 48                            | 48     |
| ACTA1    | 90.71                 | 99.47               | 100.00                          | 100.00 | 0                             | 0      |
| AGK      | 43.73                 | 36.57               | 100.00                          | 96.81  | 0                             | 53     |
| AGL      | 75.61                 | 70.07               | 100.00                          | 100.00 | 0                             | 0      |
| AGRN     | 77.01                 | 87.27               | 99.76                           | 98.63  | 17                            | 96     |
| ALG13    | 34.47                 | 43.21               | 100.00                          | 99.93  | 0                             | 3      |
| ALG14    | 54.88                 | 43.68               | 100.00                          | 100.00 | 0                             | 0      |
| ALG2     | 71.16                 | 79.95               | 100.00                          | 100.00 | 0                             | 0      |
| ANO5     | 75.71                 | 73.68               | 100.00                          | 100.00 | 0                             | 0      |
| ATP2A1   | 93.31                 | 95.29               | 100.00                          | 97.80  | 0                             | 79     |
| B3GALNT2 | 33.06                 | 25.98               | 92.04                           | 87.82  | 151                           | 231    |
| B3GNT1   | 51.99                 | 69.88               | 98.60                           | 100.00 | 18                            | 0      |
| BAG3     | 89.70                 | 91.60               | 100.00                          | 100.00 | 0                             | 0      |
| BIN1     | 83.63                 | 88.52               | 100.00                          | 100.00 | 0                             | 0      |
| C10orf2  | 96.55                 | 99.98               | 100.00                          | 100.00 | 0                             | 0      |
| CAPN3    | 90.94                 | 92.75               | 100.00                          | 100.00 | 0                             | 0      |
| CAV3     | 87.44                 | 94.75               | 100.00                          | 100.00 | 0                             | 0      |
| CCDC78   | 62.86                 | 80.17               | 100.00                          | 100.00 | 0                             | 0      |
| CFL2     | 68.21                 | 81.28               | 100.00                          | 100.00 | 0                             | 0      |
| CHAT     | 90.75                 | 93.83               | 100.00                          | 100.00 | 0                             | 0      |
| CHKB     | 91.98                 | 91.01               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 89.08                 | 93.82               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 91.20                 | 93.97               | 96.92                           | 100.00 | 56                            | 0      |
| CHRNA1   | 95.74                 | 95.79               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 89.16                 | 95.84               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 98.31                 | 99.93               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 91.10                 | 93.04               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 62.31                 | 71.95               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 71.74                 | 65.19               | 100.00                          | 100.00 | 0                             | 0      |
| COL12A1  | 44.81                 | 40.40               | 100.00                          | 99.18  | 0                             | 88     |
| COL6A1   | 86.80                 | 94.23               | 100.00                          | 100.00 | 0                             | 0      |
| COL6A2   | 87.60                 | 93.69               | 100.00                          | 100.00 | 0                             | 0      |
| COL6A3   | 86.48                 | 93.00               | 99.59                           | 99.59  | 43                            | 43     |

| Gene    | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|---------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|         | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| COLQ    | 90.89                 | 93.70               | 100.00                          | 100.00 | 0                             | 0      |
| COX15   | 95.34                 | 96.71               | 100.00                          | 100.00 | 0                             | 0      |
| CPT2    | 87.59                 | 93.28               | 100.00                          | 100.00 | 0                             | 0      |
| CRYAB   | 87.02                 | 92.10               | 100.00                          | 100.00 | 0                             | 0      |
| DAG1    | 94.02                 | 99.91               | 100.00                          | 100.00 | 0                             | 0      |
| DES     | 82.38                 | 91.62               | 100.00                          | 100.00 | 0                             | 0      |
| DMD     | 61.42                 | 74.45               | 100.00                          | 100.00 | 0                             | 0      |
| DNAJB6  | 41.09                 | 34.42               | 100.00                          | 100.00 | 0                             | 0      |
| DNM2    | 86.07                 | 86.39               | 97.80                           | 98.01  | 82                            | 74     |
| DOK7    | 72.30                 | 76.61               | 100.00                          | 97.94  | 0                             | 34     |
| DOLK    | 93.48                 | 99.98               | 100.00                          | 100.00 | 0                             | 0      |
| DPAGT1  | 93.24                 | 93.86               | 100.00                          | 100.00 | 0                             | 0      |
| DPM1    | 62.80                 | 56.38               | 93.18                           | 95.94  | 74                            | 44     |
| DPM2    | 27.88                 | 24.42               | 100.00                          | 100.00 | 0                             | 0      |
| DPM3    | 84.43                 | 94.47               | 100.00                          | 100.00 | 0                             | 0      |
| DYSF    | 92.97                 | 93.56               | 100.00                          | 100.00 | 0                             | 0      |
| EMD     | 69.98                 | 91.29               | 100.00                          | 100.00 | 0                             | 0      |
| ENO3    | 97.90                 | 99.85               | 100.00                          | 100.00 | 0                             | 0      |
| ETFA    | 53.56                 | 45.34               | 86.15                           | 86.09  | 256                           | 257    |
| ETFB    | 98.62                 | 98.90               | 100.00                          | 100.00 | 0                             | 0      |
| ETFDH   | 58.38                 | 54.95               | 100.00                          | 100.00 | 0                             | 0      |
| FAM111B | 53.15                 | 61.09               | 99.42                           | 98.48  | 13                            | 34     |
| FHL1    | 69.09                 | 85.33               | 100.00                          | 100.00 | 0                             | 0      |
| FKBP14  | 24.56                 | 18.39               | 100.00                          | 83.60  | 0                             | 121    |
| FKRP    | 55.29                 | 62.28               | 100.00                          | 97.01  | 0                             | 45     |
| FKTN    | 66.04                 | 57.09               | 97.07                           | 100.00 | 56                            | 0      |
| FLNC    | 94.92                 | 99.02               | 100.00                          | 100.00 | 0                             | 0      |
| GAA     | 84.62                 | 87.87               | 100.00                          | 100.00 | 0                             | 0      |
| GBE1    | 27.25                 | 20.10               | 100.00                          | 93.03  | 0                             | 172    |
| GFPT1   | 73.05                 | 68.53               | 100.00                          | 100.00 | 0                             | 0      |
| GMPPB   | 63.96                 | 77.47               | 100.00                          | 100.00 | 0                             | 0      |
| GNE     | 83.70                 | 78.87               | 100.00                          | 100.00 | 0                             | 0      |
| GYG1    | 77.76                 | 81.74               | 100.00                          | 100.00 | 0                             | 0      |
| GYS1    | 88.92                 | 93.14               | 100.00                          | 100.00 | 0                             | 0      |
| HNRNPDL | 39.25                 | 35.97               | 90.00                           | 91.14  | 140                           | 124    |
| IGHMBP2 | 90.87                 | 96.05               | 100.00                          | 100.00 | 0                             | 0      |

| Gene    | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|---------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|         | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| ISCU    | 69.30                 | 65.59               | 100.00                          | 98.19  | 0                             | 16     |
| ISPD    | 32.81                 | 26.93               | 82.16                           | 78.49  | 277                           | 334    |
| ITGA7   | 90.12                 | 92.14               | 100.00                          | 100.00 | 0                             | 0      |
| KBTBD13 | 49.11                 | 56.82               | 96.77                           | 99.35  | 45                            | 9      |
| KCNJ2   | 99.05                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| KLHL40  | 71.73                 | 85.65               | 100.00                          | 100.00 | 0                             | 0      |
| KLHL41  | 53.63                 | 61.95               | 100.00                          | 100.00 | 0                             | 0      |
| KLHL9   | 80.33                 | 93.22               | 100.00                          | 100.00 | 0                             | 0      |
| LAMA2   | 85.80                 | 82.89               | 100.00                          | 100.00 | 0                             | 0      |
| LAMB2   | 97.05                 | 99.84               | 100.00                          | 100.00 | 0                             | 0      |
| LAMP2   | 50.06                 | 60.68               | 100.00                          | 99.83  | 0                             | 3      |
| LARGE   | 90.15                 | 89.95               | 100.00                          | 100.00 | 0                             | 0      |
| LDB3    | 89.95                 | 95.63               | 100.00                          | 100.00 | 0                             | 0      |
| LDHA    | 70.61                 | 61.58               | 100.00                          | 100.00 | 0                             | 0      |
| LIMS2   | 55.94                 | 59.56               | 90.90                           | 90.90  | 134                           | 134    |
| LMNA    | 88.12                 | 90.58               | 100.00                          | 100.00 | 0                             | 0      |
| LPIN1   | 76.22                 | 78.12               | 100.00                          | 100.00 | 0                             | 0      |
| LRP4    | 95.67                 | 96.78               | 100.00                          | 99.68  | 0                             | 21     |
| MATR3   | 67.55                 | 75.30               | 96.08                           | 95.54  | 123                           | 140    |
| MEGF10  | 46.71                 | 39.46               | 100.00                          | 100.00 | 0                             | 0      |
| MSTN    | 72.08                 | 78.39               | 100.00                          | 100.00 | 0                             | 0      |
| MTM1    | 54.57                 | 62.60               | 100.00                          | 100.00 | 0                             | 0      |
| MTMR14  | 85.21                 | 84.39               | 100.00                          | 100.00 | 0                             | 0      |
| MTTP    | 80.04                 | 77.97               | 100.00                          | 100.00 | 0                             | 0      |
| MUSK    | 52.22                 | 52.70               | 100.00                          | 99.12  | 0                             | 27     |
| MYBPC3  | 50.86                 | 55.81               | 99.47                           | 100.00 | 24                            | 0      |
| MYF6    | 90.16                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| MYH14   | 68.18                 | 71.21               | 100.00                          | 100.00 | 0                             | 0      |
| MYH2    | 91.34                 | 95.18               | 100.00                          | 100.00 | 0                             | 0      |
| MYH3    | 95.96                 | 97.77               | 100.00                          | 99.98  | 0                             | 1      |
| MYH7    | 97.49                 | 99.08               | 100.00                          | 100.00 | 0                             | 0      |
| MYOT    | 75.98                 | 70.87               | 100.00                          | 100.00 | 0                             | 0      |
| NEB     | 45.79                 | 44.71               | 87.75                           | 90.29  | 3589                          | 2844   |
| OPA1    | 62.12                 | 57.56               | 100.00                          | 100.00 | 0                             | 0      |
| ORAI1   | 83.27                 | 80.32               | 89.78                           | 86.30  | 97                            | 130    |
| PABPN1  | 68.54                 | 67.89               | 92.39                           | 88.92  | 81                            | 118    |

| Gene     | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|----------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|          | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| PFKM     | 85.41                 | 84.15               | 100.00                          | 100.00 | 0                             | 0      |
| PGAM2    | 99.17                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| PGK1     | 77.92                 | 96.76               | 100.00                          | 100.00 | 0                             | 0      |
| PGM1     | 91.24                 | 92.32               | 100.00                          | 100.00 | 0                             | 0      |
| PHKA1    | 66.18                 | 80.50               | 100.00                          | 100.00 | 0                             | 0      |
| PLEC     | 85.21                 | 94.42               | 99.77                           | 99.77  | 36                            | 36     |
| PNPLA2   | 78.73                 | 81.78               | 99.59                           | 96.28  | 7                             | 63     |
| POLG     | 91.70                 | 93.95               | 99.00                           | 99.00  | 42                            | 42     |
| POLG2    | 67.30                 | 71.44               | 100.00                          | 100.00 | 0                             | 0      |
| POMGNT1  | 90.97                 | 93.94               | 100.00                          | 100.00 | 0                             | 0      |
| POMGNT2  | 82.97                 | 93.85               | 100.00                          | 100.00 | 0                             | 0      |
| POMK     | 81.71                 | 87.75               | 100.00                          | 100.00 | 0                             | 0      |
| POMT1    | 93.06                 | 93.43               | 100.00                          | 100.00 | 0                             | 0      |
| POMT2    | 90.34                 | 92.13               | 100.00                          | 100.00 | 0                             | 0      |
| PREPL    | 45.05                 | 38.89               | 99.96                           | 99.96  | 1                             | 1      |
| PRKAG2   | 75.44                 | 71.79               | 100.00                          | 99.91  | 0                             | 2      |
| PTPLA    | 22.56                 | 16.63               | 97.86                           | 88.89  | 27                            | 140    |
| PTRF     | 84.71                 | 91.83               | 99.34                           | 100.00 | 8                             | 0      |
| PYGM     | 98.79                 | 99.48               | 100.00                          | 100.00 | 0                             | 0      |
| RAPSN    | 84.64                 | 87.33               | 100.00                          | 100.00 | 0                             | 0      |
| RRM2B    | 68.60                 | 66.76               | 91.25                           | 91.25  | 141                           | 141    |
| RYR1     | 91.80                 | 93.54               | 97.64                           | 97.71  | 412                           | 401    |
| SCN4A    | 74.92                 | 78.25               | 100.00                          | 100.00 | 0                             | 0      |
| SEPN1    | 80.43                 | 79.99               | 89.90                           | 90.00  | 205                           | 203    |
| SGCA     | 88.28                 | 93.67               | 100.00                          | 100.00 | 0                             | 0      |
| SGCB     | 68.02                 | 55.38               | 99.40                           | 95.44  | 7                             | 53     |
| SGCD     | 37.72                 | 28.58               | 100.00                          | 100.00 | 0                             | 0      |
| SGCG     | 86.15                 | 81.36               | 100.00                          | 100.00 | 0                             | 0      |
| SIL1     | 94.85                 | 96.56               | 100.00                          | 100.00 | 0                             | 0      |
| SLC22A5  | 92.52                 | 98.08               | 100.00                          | 100.00 | 0                             | 0      |
| SLC25A20 | 93.29                 | 92.62               | 100.00                          | 100.00 | 0                             | 0      |
| SLC25A4  | 76.10                 | 81.52               | 100.00                          | 100.00 | 0                             | 0      |
| SLC52A3  | 93.42                 | 98.85               | 100.00                          | 100.00 | 0                             | 0      |
| SMCHD1   | 24.46                 | 18.93               | 98.56                           | 91.29  | 102                           | 616    |
| STAC3    | 60.18                 | 64.21               | 100.00                          | 100.00 | 0                             | 0      |
| STIM1    | 86.56                 | 88.71               | 100.00                          | 100.00 | 0                             | 0      |

| Gene     | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|----------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|          | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| STIM2    | 43.79                 | 36.33               | 98.34                           | 90.21  | 47                            | 277    |
| SUCLA2   | 70.70                 | 57.72               | 100.00                          | 100.00 | 0                             | 0      |
| SYNE1    | 84.25                 | 83.72               | 100.00                          | 99.76  | 0                             | 71     |
| SYNE2    | 74.71                 | 76.64               | 99.98                           | 99.40  | 4                             | 146    |
| TARDBP   | 67.48                 | 68.96               | 89.56                           | 89.56  | 165                           | 165    |
| TAZ      | 81.92                 | 98.97               | 100.00                          | 100.00 | 0                             | 0      |
| TCAP     | 83.34                 | 99.08               | 100.00                          | 100.00 | 0                             | 0      |
| TIA1     | 32.56                 | 25.08               | 100.00                          | 100.00 | 0                             | 0      |
| TK2      | 77.13                 | 77.10               | 94.97                           | 95.71  | 68                            | 58     |
| TMEM43   | 74.28                 | 72.68               | 100.00                          | 100.00 | 0                             | 0      |
| TMEM5    | 31.07                 | 25.29               | 100.00                          | 99.87  | 0                             | 2      |
| TNNI2    | 93.91                 | 96.09               | 100.00                          | 100.00 | 0                             | 0      |
| TNNT1    | 69.98                 | 71.29               | 95.35                           | 98.25  | 64                            | 24     |
| TNNT3    | 99.16                 | 98.90               | 100.00                          | 100.00 | 0                             | 0      |
| TNPO3    | 44.98                 | 40.35               | 100.00                          | 99.97  | 0                             | 1      |
| TOR1AIP1 | 51.16                 | 58.75               | 97.51                           | 100.00 | 55                            | 0      |
| TPM2     | 90.27                 | 89.12               | 100.00                          | 100.00 | 0                             | 0      |
| TPM3     | 96.87                 | 96.10               | 100.00                          | 100.00 | 0                             | 0      |
| TRAPPC11 | 37.43                 | 29.89               | 100.00                          | 95.58  | 0                             | 177    |
| TRIM32   | 94.96                 | 99.52               | 100.00                          | 100.00 | 0                             | 0      |
| TTN      | 58.05                 | 63.24               | 98.35                           | 99.37  | 2002                          | 766    |
| UBA1     | 85.50                 | 93.45               | 99.98                           | 100.00 | 1                             | 0      |
| VAPB     | 80.46                 | 74.41               | 100.00                          | 100.00 | 0                             | 0      |
| VCP      | 98.26                 | 98.78               | 100.00                          | 100.00 | 0                             | 0      |
| VMA21    | 41.32                 | 58.40               | 100.00                          | 100.00 | 0                             | 0      |
| YARS2    | 83.53                 | 86.56               | 100.00                          | 100.00 | 0                             | 0      |

**Notes:**

<sup>1</sup>Average per-base coverage for gene. Genes that are not completely callable are highlighted in pink. For more information on how coverage was calculated, please refer to *General Methodology* section.

<sup>2</sup>The percent of sites that are considered callable for this gene. For more information on how callability is determined, please refer to *General Methodology* section.

<sup>3</sup>The average for samples included in the cohort.

<sup>4</sup>The metric for the sample in this report.

## patient 5

**Proband ID:** MWAR110  
**Inferred Sex (Reported):** Female (Female)  
**Inferred Ancestry (Reported):** European (European)

### Candidate Mutations

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i><br><i>Stars</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|-------------------------------------------------------------------|-------|
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i>            | 11:22291971 A>G<br>c.2012A>G<br><b>p.Tyr671Cys</b>                                                                       | 8.24e-06<br>1.50e-05                                    | NA                                                                |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i>            | 11:22242646 C>CA<br>c.191dupA<br><b>p.Asn64LysfsTer15</b>                                                                | 1.03e-03<br>1.71e-03                                    | NA                                                                |       |

#### Footnotes:

<sup>1</sup> Genomic variants are based on build37/hg19. This is a filtered list of variants containing **nonsense, frameshift, essential splice** and **missense** variants.

<sup>2</sup> ExAC Allele Frequency (AF). For more information on ExAC please refer to *General Methodology* section.

<sup>3</sup> For more details on ClinVar and how confidence is rated please refer to *General Methodology* section.

<sup>4</sup> Genotype is either heterozygous (*het*), homozygous (*hom*) or hemizygous (*hem*) for the variant.

<sup>5</sup> Transcript changes are based on Ensembl.

<sup>6</sup> Highest allele frequency in any of the given ExAC populations (Pop).

<sup>7</sup> rsID if present in dbSNP version 141.

<sup>+</sup> Large insertion/deletions have been truncated.

#### Limitations:

- Analysis limited to gene list. For more information on gene list please refer to *Appendix*.
- This exome was sequenced in a research environment and results have not been clinically validated. Clinical validation of candidate causal variants should be performed prior to making a formal clinical diagnosis. Please refer to *Appendix* for coverage of this sample.
- Proband only approach used in this project and thus unable to determine phasing of two or more variants (whether in *cis* or *trans*) and also unable to determine if mutations are inherited or de novo. Determining phasing and inheritance of variants requires sequencing of parents.

## Appendix - Candidate Gene List

### All Rare Variants Called

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------|
| <b>ABHD5</b><br>ENST00000458276.2<br><i>het</i>           | 3:43756577 C>CTG<br>c.773+61_773+62dupGT                                                                                 | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127943 A>C<br>c.1748-18A>C                                                                                           | 1.08e-03<br>7.52e-03                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127948 A>C<br>c.1748-13A>C                                                                                           | 5.60e-04<br>6.34e-03                                    | NA                                                |       |
| <b>ACADVL</b><br>ENST00000543245.2<br><i>het</i>          | 17:7127951 T>C<br>c.1748-10T>C                                                                                           | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>AGRN</b><br>ENST00000379370.2<br><i>het</i>            | 1:982302 C>A<br>c.3353C>A<br>p.Thr1118Lys<br>rs149159118                                                                 | 2.15e-03<br>1.43e-02                                    | NA                                                |       |
| <b>ALG13</b><br>ENST00000394780.3<br><i>het</i>           | X:110987996 ACCT>A<br>c.2833_2835delCCT<br>p.Pro945del                                                                   | 7.11e-03<br>1.55e-02                                    | NA                                                |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i>            | 11:22242646 C>CA<br>c.191dupA<br>p.Asn64LysfsTer15                                                                       | 1.03e-03<br>1.71e-03                                    | NA                                                |       |
| <b>ANO5</b><br>ENST00000324559.8<br><i>het</i>            | 11:22291971 A>G<br>c.2012A>G<br>p.Tyr671Cys                                                                              | 8.24e-06<br>1.50e-05                                    | NA                                                |       |
| <b>AR</b><br>ENST00000374690.3<br><i>het</i>              | X:66765158 TGCA>T<br>c.237_239delGCA<br>p.Gln80del                                                                       | 3.15e-03<br>6.12e-03                                    | NA                                                |       |

| Gene<br><i>Transcript</i><br><i>Genotype<sup>4</sup></i> | Gene Variant <sup>1</sup><br><i>Transcript Change<sup>5</sup></i><br><i>Protein Change</i><br><i>dbSNP<sup>7</sup></i> | ExAC AF <sup>2</sup><br><i>Highest Pop<sup>6</sup></i> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|----------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------|-------|
|                                                          |                                                                                                                        |                                                        |                                                   |       |
| <b>CAPN3</b><br>ENST00000397163.3<br><i>het</i>          | 15:42684803 G>A<br>c.946-34G>A                                                                                         | 1.39e-04<br>3.34e-04                                   | NA                                                |       |
| <b>CAPN3</b><br>ENST00000397163.3<br><i>het</i>          | 15:42695248 C>A<br>c.1745+48C>A                                                                                        | 1.89e-04<br>1.22e-03                                   | NA                                                |       |
| <b>CLCN1</b><br>ENST00000343257.2<br><i>het</i>          | 7:143039510 G>C<br>c.1842G>C<br><b>p.Lys614Asn</b><br>rs140205115                                                      | 1.38e-03<br>2.16e-03                                   | NA                                                |       |
| <b>COL6A1</b><br>ENST00000361866.3<br><i>het</i>         | 21:47410376 A>AG<br>c.1002+40_1002+41insG                                                                              | 2.72e-04<br>1.89e-03                                   | NA                                                |       |
| <b>COL6A1</b><br>ENST00000361866.3<br><i>het</i>         | 21:47410379 A>ACGGGGAGGG <sup>+</sup><br>c.1002+43_1002+44insCGGGGAGGGA <sup>+</sup>                                   | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>COL6A2</b><br>ENST00000300527.4<br><i>het</i>         | 21:47532521 C>T<br>c.714+30C>T                                                                                         | 2.95e-04<br>4.92e-04                                   | NA                                                |       |
| <b>DMD</b><br><br><i>het</i>                             | X:31132813 T>G                                                                                                         | 1.57e-04<br>4.16e-04                                   | NA                                                |       |
| <b>DMPK</b><br>ENST00000343373.4<br><i>het</i>           | 19:46274987 ACCGGCCCGC <sup>+</sup> >A<br>c.1532+30_1532+41delGATGCGGGCC <sup>+</sup>                                  | 5.48e-04<br>9.41e-04                                   | NA                                                |       |
| <b>DYSF</b><br>ENST00000410020.3<br><i>het</i>           | 2:71780887 A>C<br>c.1985-50A>C                                                                                         | 7.18e-04<br>3.66e-03                                   | NA                                                |       |
| <b>DYSF</b><br>ENST00000410020.3                         | 2:71780891 G>C<br>c.1985-46G>C                                                                                         | 4.36e-04<br>1.73e-03                                   | NA                                                |       |

| Gene<br>Transcript<br>Genotype <sup>4</sup>       | Gene Variant <sup>1</sup><br>Transcript Change <sup>5</sup><br>Protein Change<br>dbSNP <sup>7</sup> | ExAC AF <sup>2</sup><br>Highest Pop <sup>6</sup> | ClinVar <sup>3</sup><br>ClinVar Confidence | Notes |
|---------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------------------------|--------------------------------------------|-------|
| <i>het</i>                                        |                                                                                                     |                                                  |                                            |       |
| <b>DYSF</b><br>ENST00000410020.3<br><i>het</i>    | 2:71780895 T>C<br>c.1985-42T>C                                                                      | 0.00e+00<br>0.00e+00                             | NA                                         |       |
| <b>DYSF</b><br>ENST00000410020.3<br><i>het</i>    | 2:71780898 A>C<br>c.1985-39A>C                                                                      | 0.00e+00<br>0.00e+00                             | NA                                         |       |
| <b>DYSF</b><br>ENST00000410020.3<br><i>het</i>    | 2:71780900 T>C<br>c.1985-37T>C                                                                      | 0.00e+00<br>0.00e+00                             | NA                                         |       |
| <b>FLNC</b><br>ENST00000325888.8<br><i>het</i>    | 7:128482640 C>T<br>c.2277C>T<br>c.2277C>T(p.=)                                                      | 0.00e+00<br>0.00e+00                             | NA                                         |       |
| <b>IGHMBP2</b><br>ENST00000255078.3<br><i>het</i> | 11:68673601 C>G<br>c.151C>G<br>p.Gln51Glu<br>rs117061430                                            | 6.28e-03<br>1.44e-02                             | NA                                         |       |
| <b>ITGA7</b><br>ENST00000257880.7<br><i>het</i>   | 12:56080043 C>T<br>c.3371G>A<br>p.Arg1124Gln<br>rs201332795                                         | 4.01e-04<br>6.55e-04                             | NA                                         |       |
| <b>ITGA7</b><br>ENST00000553804.1<br><i>het</i>   | 12:56086993 C>T<br>c.2656G>A<br>p.Glu886Lys<br>rs144983062                                          | 4.01e-03<br>6.03e-03                             | NA                                         |       |
| <b>KBTBD13</b><br>ENST00000432196.2<br><i>het</i> | 15:65369514 G>A<br>c.361G>A<br>p.Val121Met<br>rs201466173                                           | 7.09e-04<br>4.85e-03                             | NA                                         |       |
| <b>LAMB2</b><br>ENST00000418109.1<br><i>het</i>   | 3:49162352 C>T<br>c.2891G>A<br>p.Arg964Gln                                                          | 2.48e-05<br>2.60e-04                             | NA                                         |       |
| <b>LDB3</b>                                       | 10:88439092 A>G                                                                                     | 3.30e-05                                         | NA                                         |       |

| Gene<br><i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | Gene Variant <sup>1</sup><br><i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | ExAC AF <sup>2</sup><br><i>Highest Pop</i> <sup>6</sup> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------|
| ENST00000429277.2<br><i>het</i>                           | c.94-32A>G                                                                                                               | 2.90e-04                                                |                                                   |       |
| <b>LDB3</b><br>ENST00000429277.2<br><i>het</i>            | 10:88451715 A>G<br>c.956A>G<br><b>p.Lys319Arg</b>                                                                        | 6.65e-03<br>4.84e-02                                    | NA                                                |       |
| <b>LDB3</b><br>ENST00000429277.2<br><i>het</i>            | 10:88478529 G>A<br>c.1918G>A<br><b>p.Val640Ile</b><br>rs45618633                                                         | 6.99e-03<br>6.37e-02                                    | NA                                                |       |
| <b>MYH3</b><br>ENST00000583535.1<br><i>het</i>            | 17:10555926 CTTT>C<br>c.205-49_205-47delIAAA                                                                             | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>MYH3</b><br>ENST00000583535.1<br><i>het</i>            | 17:10555926 CTTTTT>C<br>c.205-51_205-47delIAAAAA                                                                         | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>MYH3</b><br>ENST00000583535.1<br><i>het</i>            | 17:10555932 T>C<br>c.205-52A>G                                                                                           | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>NEB</b><br>ENST00000397345.3<br><i>het</i>             | 2:152381168 CTTT>C<br>c.22273-41_22273-39delIAAA                                                                         | 0.00e+00<br>0.00e+00                                    | NA                                                |       |
| <b>NEB</b><br>ENST00000397345.3<br><i>het</i>             | 2:152381170 T>C<br>c.22273-40A>G                                                                                         | 2.76e-05<br>2.37e-04                                    | NA                                                |       |
| <b>NEB</b><br>ENST00000397345.3<br><i>het</i>             | 2:152381172 T>C<br>c.22273-42A>G                                                                                         | 1.09e-05<br>9.79e-05                                    | NA                                                |       |
| <b>NEB</b><br>ENST00000397345.3<br><i>het</i>             | 2:152456944 G>C<br>c.13788+12C>G                                                                                         | 0.00e+00<br>0.00e+00                                    | NA                                                |       |

| Gene<br><i>Transcript<br/>Genotype<sup>4</sup></i> | Gene Variant <sup>1</sup><br><i>Transcript Change<sup>5</sup><br/>Protein Change<br/>dbSNP<sup>7</sup></i> | ExAC AF <sup>2</sup><br><i>Highest Pop<sup>6</sup></i> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|----------------------------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------|-------|
| <b>NEB</b><br>ENST00000397345.3<br><i>het</i>      | 2:152457023 T>C<br>c.13721A>G<br><b>p.His4574Arg</b>                                                       | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>PFKM</b><br>ENST00000340802.6<br><i>het</i>     | 12:48501129 ACT>A<br>c.-9-16_-9-15delCT                                                                    | 1.99e-03<br>2.73e-03                                   | NA                                                |       |
| <b>PHKA1</b><br>ENST00000373542.4<br><i>het</i>    | X:71813192 T>C<br>c.3073-68A>G                                                                             | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>POMGNT2</b><br>ENST00000344697.2<br><i>het</i>  | 3:43122465 G>A<br>c.459C>T<br><b>c.459C&gt;T(p.=)</b>                                                      | 1.40e-04<br>4.32e-04                                   | NA                                                |       |
| <b>PTPLA</b><br>ENST00000361271.3<br><i>het</i>    | 10:17646048 T>TA<br>c.258-3dupT                                                                            | 4.11e-03<br>1.88e-02                                   | NA                                                |       |
| <b>RYR1</b><br>ENST00000359596.3<br><i>het</i>     | 19:38976331 G>A<br>c.5036G>A<br><b>p.Arg1679His</b><br>rs146504767                                         | 1.53e-03<br>2.63e-03                                   | NA                                                |       |
| <b>SCN4A</b><br>ENST00000435607.1<br><i>het</i>    | 17:62038535 T>C<br>c.1845+18A>G                                                                            | 2.39e-03<br>4.07e-03                                   | NA                                                |       |
| <b>SCN4A</b><br>ENST00000435607.1<br><i>het</i>    | 17:62049196 C>T<br>c.497G>A<br><b>p.Gly166Glu</b>                                                          | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>SCN4A</b><br>ENST00000435607.1<br><i>het</i>    | 17:62049198 T>G<br>c.495A>C<br><b>c.495A&gt;C(p.=)</b>                                                     | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>STIM2</b><br>ENST00000465503.1<br><i>het</i>    | 4:27004500 T>A<br>c.804-49T>A                                                                              | 1.85e-03<br>1.07e-02                                   | NA                                                |       |

| Gene<br><i>Transcript<br/>Genotype<sup>4</sup></i> | Gene Variant <sup>1</sup><br><i>Transcript Change<sup>5</sup><br/>Protein Change<br/>dbSNP<sup>7</sup></i> | ExAC AF <sup>2</sup><br><i>Highest Pop<sup>6</sup></i> | ClinVar <sup>3</sup><br><i>ClinVar Confidence</i> | Notes |
|----------------------------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------|-------|
|                                                    |                                                                                                            |                                                        |                                                   |       |
| <b>TNNT1</b><br>ENST00000588981.1<br><i>het</i>    | 19:55646893 C>CTT<br>c.612-1323.612-1322dupAA                                                              | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179419925 G>GA<br>c.88307-47dupT                                                                         | 3.19e-03<br>1.32e-02                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179517639 C>T<br>c.38895G>A<br>c.38895G>A(p.=)                                                           | 8.55e-06<br>6.08e-05                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179519303 T>C<br>c.38207-42A>G                                                                           | 3.33e-03<br>1.94e-02                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179553730 AT>A<br>c.32095+49delA                                                                         | 6.92e-04<br>2.29e-03                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179553730 ATT>A<br>c.32095+48_32095+49delAA                                                              | 1.41e-03<br>3.44e-03                                   | NA                                                |       |
| <b>TTN</b><br>ENST00000589042.1<br><i>het</i>      | 2:179634389 TATA>T<br>c.8902+14_8902+16delTAT                                                              | 5.72e-04<br>1.62e-03                                   | NA                                                |       |
| <b>UBA1</b><br>ENST00000335972.6<br><i>het</i>     | X:47063147 T>C<br>c.1575+52T>C                                                                             | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>YARS2</b><br>ENST00000324868.8<br><i>het</i>    | 12:32905674 C>CAAA<br>c.947+1175_947+1177dupTTT                                                            | 0.00e+00<br>0.00e+00                                   | NA                                                |       |
| <b>YARS2</b><br>ENST00000324868.8                  | 12:32905674 CA>C<br>c.947+1177delT                                                                         | 0.00e+00<br>0.00e+00                                   | NA                                                |       |

| Gene                                              | Gene Variant <sup>1</sup>                                                                   | ExAC AF <sup>2</sup>            | ClinVar <sup>3</sup>      | Notes |
|---------------------------------------------------|---------------------------------------------------------------------------------------------|---------------------------------|---------------------------|-------|
| <i>Transcript</i><br><i>Genotype</i> <sup>4</sup> | <i>Transcript Change</i> <sup>5</sup><br><i>Protein Change</i><br><i>dbSNP</i> <sup>7</sup> | <i>Highest Pop</i> <sup>6</sup> | <i>ClinVar Confidence</i> |       |
| <i>het</i>                                        |                                                                                             |                                 |                           |       |

**Footnotes:**

<sup>1</sup> Genomic variants are based on build37/hg19. This is a filtered list (AF <0.01) of variants containing **non-sense, frameshift, essential splice, missense** and **synonymous** variants.

<sup>2</sup> ExAC Allele Frequency (AF). For more information on ExAC please refer to *General Methodology* section.

<sup>3</sup> For more details on ClinVar and how confidence is rated please refer to *General Methodology* section.

<sup>4</sup> Genotype is either heterozygous (*het*), homozygous (*hom*) or hemizygous (*hem*) for the variant.

<sup>5</sup> Transcript changes are based on Ensembl.

<sup>6</sup> Highest allele frequency in any of the given ExAC populations (Pop).

<sup>7</sup> rsID if present in dbSNP version 141.

<sup>+</sup> Large insertion/deletions have been truncated.

## Gene Coverage

| Gene     | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|----------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|          | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| ABHD5    | 83.68                 | 91.38               | 100.00                          | 94.44  | 0                             | 67     |
| ACADS    | 94.54                 | 98.13               | 100.00                          | 100.00 | 0                             | 0      |
| ACADVL   | 93.76                 | 97.78               | 98.24                           | 99.45  | 48                            | 15     |
| ACTA1    | 93.53                 | 99.78               | 100.00                          | 100.00 | 0                             | 0      |
| AGK      | 45.08                 | 45.21               | 100.00                          | 100.00 | 0                             | 0      |
| AGL      | 78.86                 | 90.31               | 100.00                          | 100.00 | 0                             | 0      |
| AGRN     | 81.32                 | 94.95               | 99.10                           | 96.55  | 63                            | 241    |
| ALG13    | 36.96                 | 56.04               | 100.00                          | 100.00 | 0                             | 0      |
| ALG14    | 54.11                 | 61.88               | 100.00                          | 100.00 | 0                             | 0      |
| ALG2     | 75.43                 | 92.00               | 100.00                          | 100.00 | 0                             | 0      |
| ANO5     | 77.61                 | 67.02               | 100.00                          | 100.00 | 0                             | 0      |
| ATP2A1   | 94.12                 | 97.40               | 100.00                          | 100.00 | 0                             | 0      |
| B3GALNT2 | 34.38                 | 55.20               | 93.04                           | 96.42  | 132                           | 68     |
| B3GNT1   | 58.75                 | 90.00               | 99.22                           | 100.00 | 10                            | 0      |
| BAG3     | 89.48                 | 89.96               | 100.00                          | 99.51  | 0                             | 9      |
| BIN1     | 84.84                 | 91.92               | 100.00                          | 100.00 | 0                             | 0      |
| C10orf2  | 97.85                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| CAPN3    | 91.79                 | 96.82               | 100.00                          | 100.00 | 0                             | 0      |
| CAV3     | 89.42                 | 95.57               | 100.00                          | 100.00 | 0                             | 0      |
| CCDC78   | 73.79                 | 98.63               | 100.00                          | 100.00 | 0                             | 0      |
| CFL2     | 77.81                 | 96.75               | 100.00                          | 100.00 | 0                             | 0      |
| CHAT     | 92.00                 | 98.28               | 100.00                          | 100.00 | 0                             | 0      |
| CHKB     | 89.99                 | 90.83               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 92.36                 | 99.90               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 93.26                 | 99.79               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 96.33                 | 99.69               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 92.21                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 98.84                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 92.66                 | 98.80               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 71.89                 | 99.30               | 100.00                          | 100.00 | 0                             | 0      |
| CHRNA1   | 74.15                 | 67.12               | 100.00                          | 100.00 | 0                             | 0      |
| COL12A1  | 46.85                 | 45.11               | 100.00                          | 99.97  | 0                             | 3      |
| COL6A1   | 90.04                 | 98.94               | 100.00                          | 100.00 | 0                             | 0      |
| COL6A2   | 90.63                 | 98.66               | 100.00                          | 100.00 | 0                             | 0      |
| COL6A3   | 90.25                 | 99.07               | 99.59                           | 99.59  | 43                            | 43     |

| Gene        | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |              | Uncallable Sites <sup>2</sup> |            |
|-------------|-----------------------|---------------------|---------------------------------|--------------|-------------------------------|------------|
|             | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample       | Cohort                        | Sample     |
| COLQ        | 92.21                 | 97.68               | 100.00                          | 100.00       | 0                             | 0          |
| COX15       | 96.39                 | 99.77               | 100.00                          | 100.00       | 0                             | 0          |
| CPT2        | 90.21                 | 99.51               | 100.00                          | 100.00       | 0                             | 0          |
| CRYAB       | 90.19                 | 99.07               | 100.00                          | 100.00       | 0                             | 0          |
| DAG1        | 96.25                 | 99.99               | 100.00                          | 100.00       | 0                             | 0          |
| DES         | 84.69                 | 96.23               | 100.00                          | 100.00       | 0                             | 0          |
| DMD         | 62.89                 | 72.07               | 100.00                          | 100.00       | 0                             | 0          |
| DNAJB6      | 43.18                 | 69.43               | 100.00                          | 100.00       | 0                             | 0          |
| <b>DNM2</b> | <b>86.20</b>          | <b>89.63</b>        | <b>98.66</b>                    | <b>97.32</b> | <b>50</b>                     | <b>100</b> |
| DOK7        | 73.98                 | 87.13               | 100.00                          | 100.00       | 0                             | 0          |
| DOLK        | 95.96                 | 100.00              | 100.00                          | 100.00       | 0                             | 0          |
| DPAGT1      | 94.40                 | 99.63               | 100.00                          | 100.00       | 0                             | 0          |
| DPM1        | 67.41                 | 89.93               | 95.94                           | 100.00       | 44                            | 0          |
| DPM2        | 31.60                 | 57.71               | 100.00                          | 100.00       | 0                             | 0          |
| DPM3        | 88.01                 | 100.00              | 100.00                          | 100.00       | 0                             | 0          |
| DYSF        | 92.98                 | 96.42               | 100.00                          | 100.00       | 0                             | 0          |
| EMD         | 74.97                 | 99.83               | 100.00                          | 100.00       | 0                             | 0          |
| ENO3        | 98.64                 | 100.00              | 100.00                          | 100.00       | 0                             | 0          |
| <b>ETFA</b> | <b>55.54</b>          | <b>74.07</b>        | <b>87.45</b>                    | <b>89.12</b> | <b>232</b>                    | <b>201</b> |
| ETFB        | 98.36                 | 99.89               | 100.00                          | 100.00       | 0                             | 0          |
| ETFDH       | 63.28                 | 85.94               | 100.00                          | 100.00       | 0                             | 0          |
| FAM111B     | 63.64                 | 83.81               | 100.00                          | 100.00       | 0                             | 0          |
| FHL1        | 72.44                 | 91.36               | 100.00                          | 100.00       | 0                             | 0          |
| FKBP14      | 28.20                 | 48.16               | 100.00                          | 100.00       | 0                             | 0          |
| FKRP        | 57.88                 | 74.97               | 100.00                          | 100.00       | 0                             | 0          |
| FKTN        | 67.28                 | 75.96               | 100.00                          | 100.00       | 0                             | 0          |
| FLNC        | 96.59                 | 99.99               | 100.00                          | 100.00       | 0                             | 0          |
| GAA         | 86.99                 | 96.29               | 100.00                          | 100.00       | 0                             | 0          |
| <b>GBE1</b> | <b>26.86</b>          | <b>24.02</b>        | <b>100.00</b>                   | <b>99.39</b> | <b>0</b>                      | <b>15</b>  |
| GFPT1       | 76.75                 | 96.22               | 100.00                          | 100.00       | 0                             | 0          |
| GMPPB       | 74.62                 | 96.58               | 100.00                          | 100.00       | 0                             | 0          |
| GNE         | 86.65                 | 96.26               | 100.00                          | 100.00       | 0                             | 0          |
| GYG1        | 83.01                 | 98.16               | 100.00                          | 100.00       | 0                             | 0          |
| GYS1        | 90.90                 | 98.75               | 100.00                          | 100.00       | 0                             | 0          |
| HNRNPDL     | 46.08                 | 76.51               | 91.43                           | 100.00       | 120                           | 0          |
| IGHMBP2     | 93.09                 | 99.35               | 100.00                          | 100.00       | 0                             | 0          |

| Gene    | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|---------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|         | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| ISCU    | 72.53                 | 83.38               | 100.00                          | 100.00 | 0                             | 0      |
| ISPD    | 34.20                 | 33.65               | 82.16                           | 82.16  | 277                           | 277    |
| ITGA7   | 91.25                 | 97.19               | 100.00                          | 100.00 | 0                             | 0      |
| KBTBD13 | 52.77                 | 87.36               | 100.00                          | 100.00 | 0                             | 0      |
| KCNJ2   | 99.39                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| KLHL40  | 77.98                 | 95.74               | 100.00                          | 100.00 | 0                             | 0      |
| KLHL41  | 60.50                 | 79.21               | 100.00                          | 100.00 | 0                             | 0      |
| KLHL9   | 86.83                 | 98.36               | 100.00                          | 100.00 | 0                             | 0      |
| LAMA2   | 86.67                 | 80.82               | 100.00                          | 100.00 | 0                             | 0      |
| LAMB2   | 98.07                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| LAMP2   | 53.21                 | 85.48               | 100.00                          | 100.00 | 0                             | 0      |
| LARGE   | 90.79                 | 96.74               | 100.00                          | 100.00 | 0                             | 0      |
| LDB3    | 91.97                 | 99.12               | 100.00                          | 100.00 | 0                             | 0      |
| LDHA    | 74.39                 | 92.98               | 100.00                          | 100.00 | 0                             | 0      |
| LIMS2   | 57.87                 | 75.20               | 90.90                           | 90.90  | 134                           | 134    |
| LMNA    | 89.36                 | 96.77               | 100.00                          | 100.00 | 0                             | 0      |
| LPIN1   | 79.87                 | 94.80               | 100.00                          | 100.00 | 0                             | 0      |
| LRP4    | 96.39                 | 98.94               | 100.00                          | 99.44  | 0                             | 36     |
| MATR3   | 74.40                 | 94.80               | 95.54                           | 98.34  | 140                           | 52     |
| MEGF10  | 47.32                 | 47.64               | 100.00                          | 100.00 | 0                             | 0      |
| MSTN    | 76.55                 | 72.92               | 100.00                          | 100.00 | 0                             | 0      |
| MTM1    | 58.61                 | 83.02               | 100.00                          | 100.00 | 0                             | 0      |
| MTMR14  | 85.73                 | 93.50               | 100.00                          | 100.00 | 0                             | 0      |
| MTTP    | 81.84                 | 78.70               | 100.00                          | 100.00 | 0                             | 0      |
| MUSK    | 53.15                 | 51.15               | 100.00                          | 100.00 | 0                             | 0      |
| MYBPC3  | 53.17                 | 77.93               | 100.00                          | 100.00 | 0                             | 0      |
| MYF6    | 93.63                 | 96.92               | 100.00                          | 100.00 | 0                             | 0      |
| MYH14   | 67.45                 | 83.07               | 100.00                          | 100.00 | 0                             | 0      |
| MYH2    | 93.52                 | 99.41               | 100.00                          | 100.00 | 0                             | 0      |
| MYH3    | 97.23                 | 99.83               | 100.00                          | 99.98  | 0                             | 1      |
| MYH7    | 98.21                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| MYOT    | 79.74                 | 94.22               | 100.00                          | 100.00 | 0                             | 0      |
| NEB     | 47.84                 | 52.33               | 90.81                           | 93.91  | 2694                          | 1784   |
| OPA1    | 66.67                 | 82.41               | 100.00                          | 100.00 | 0                             | 0      |
| ORAI1   | 80.83                 | 82.17               | 89.78                           | 94.42  | 97                            | 53     |
| PABPN1  | 68.47                 | 75.87               | 91.92                           | 91.83  | 86                            | 87     |

| Gene     | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|----------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|          | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| PFKM     | 85.99                 | 89.45               | 100.00                          | 100.00 | 0                             | 0      |
| PGAM2    | 99.43                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| PGK1     | 81.41                 | 99.77               | 100.00                          | 100.00 | 0                             | 0      |
| PGM1     | 92.24                 | 94.67               | 100.00                          | 100.00 | 0                             | 0      |
| PHKA1    | 67.97                 | 88.56               | 100.00                          | 100.00 | 0                             | 0      |
| PLEC     | 88.29                 | 98.37               | 99.77                           | 99.77  | 36                            | 36     |
| PNPLA2   | 80.60                 | 89.14               | 99.41                           | 99.82  | 10                            | 3      |
| POLG     | 93.11                 | 98.98               | 100.00                          | 100.00 | 0                             | 0      |
| POLG2    | 71.99                 | 90.31               | 100.00                          | 100.00 | 0                             | 0      |
| POMGNT1  | 92.89                 | 98.82               | 100.00                          | 100.00 | 0                             | 0      |
| POMGNT2  | 88.11                 | 99.70               | 100.00                          | 100.00 | 0                             | 0      |
| POMK     | 87.21                 | 99.75               | 100.00                          | 100.00 | 0                             | 0      |
| POMT1    | 94.51                 | 99.17               | 100.00                          | 100.00 | 0                             | 0      |
| POMT2    | 91.63                 | 98.73               | 100.00                          | 100.00 | 0                             | 0      |
| PREPL    | 49.65                 | 58.35               | 100.00                          | 99.96  | 0                             | 1      |
| PRKAG2   | 77.18                 | 89.07               | 100.00                          | 96.17  | 0                             | 90     |
| PTPLA    | 24.92                 | 45.61               | 99.52                           | 98.81  | 6                             | 15     |
| PTRF     | 88.09                 | 97.57               | 100.00                          | 100.00 | 0                             | 0      |
| PYGM     | 98.99                 | 99.92               | 100.00                          | 100.00 | 0                             | 0      |
| RAPSN    | 85.59                 | 96.28               | 100.00                          | 100.00 | 0                             | 0      |
| RRM2B    | 71.90                 | 83.82               | 91.25                           | 91.25  | 141                           | 141    |
| RYR1     | 92.09                 | 94.93               | 98.00                           | 98.74  | 350                           | 221    |
| SCN4A    | 76.72                 | 90.73               | 100.00                          | 100.00 | 0                             | 0      |
| SEPN1    | 80.21                 | 83.85               | 90.00                           | 89.95  | 203                           | 204    |
| SGCA     | 90.26                 | 95.06               | 100.00                          | 100.00 | 0                             | 0      |
| SGCB     | 69.82                 | 86.46               | 95.44                           | 95.44  | 53                            | 53     |
| SGCD     | 35.63                 | 28.20               | 100.00                          | 100.00 | 0                             | 0      |
| SGCG     | 87.47                 | 99.35               | 100.00                          | 100.00 | 0                             | 0      |
| SIL1     | 95.68                 | 98.55               | 100.00                          | 100.00 | 0                             | 0      |
| SLC22A5  | 93.44                 | 99.46               | 100.00                          | 100.00 | 0                             | 0      |
| SLC25A20 | 94.03                 | 96.85               | 100.00                          | 100.00 | 0                             | 0      |
| SLC25A4  | 78.74                 | 87.86               | 100.00                          | 96.76  | 0                             | 33     |
| SLC52A3  | 95.19                 | 99.84               | 100.00                          | 100.00 | 0                             | 0      |
| SMCHD1   | 28.12                 | 41.38               | 98.09                           | 96.53  | 135                           | 245    |
| STAC3    | 65.39                 | 87.12               | 100.00                          | 100.00 | 0                             | 0      |
| STIM1    | 88.16                 | 96.49               | 100.00                          | 100.00 | 0                             | 0      |

| Gene     | Coverage <sup>1</sup> |                     | Callable Sites <sup>2</sup> (%) |        | Uncallable Sites <sup>2</sup> |        |
|----------|-----------------------|---------------------|---------------------------------|--------|-------------------------------|--------|
|          | Cohort <sup>3</sup>   | Sample <sup>4</sup> | Cohort                          | Sample | Cohort                        | Sample |
| STIM2    | 44.95                 | 44.59               | 98.13                           | 92.65  | 53                            | 208    |
| SUCLA2   | 74.34                 | 96.62               | 100.00                          | 100.00 | 0                             | 0      |
| SYNE1    | 86.57                 | 94.08               | 100.00                          | 100.00 | 0                             | 0      |
| SYNE2    | 79.41                 | 95.81               | 100.00                          | 100.00 | 0                             | 0      |
| TARDBP   | 71.27                 | 86.81               | 89.56                           | 89.56  | 165                           | 165    |
| TAZ      | 84.39                 | 99.66               | 100.00                          | 100.00 | 0                             | 0      |
| TCAP     | 88.42                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| TIA1     | 34.83                 | 62.37               | 100.00                          | 100.00 | 0                             | 0      |
| TK2      | 77.78                 | 85.36               | 95.71                           | 97.63  | 58                            | 32     |
| TMEM43   | 76.20                 | 89.51               | 100.00                          | 100.00 | 0                             | 0      |
| TMEM5    | 35.25                 | 51.94               | 100.00                          | 100.00 | 0                             | 0      |
| TNNI2    | 95.21                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| TNNT1    | 71.84                 | 88.68               | 100.00                          | 100.00 | 0                             | 0      |
| TNNT3    | 99.13                 | 100.00              | 100.00                          | 100.00 | 0                             | 0      |
| TNPO3    | 46.54                 | 62.67               | 100.00                          | 100.00 | 0                             | 0      |
| TOR1AIP1 | 55.41                 | 71.89               | 100.00                          | 100.00 | 0                             | 0      |
| TPM2     | 92.36                 | 99.92               | 100.00                          | 100.00 | 0                             | 0      |
| TPM3     | 96.92                 | 99.27               | 100.00                          | 100.00 | 0                             | 0      |
| TRAPPC11 | 41.68                 | 65.85               | 100.00                          | 100.00 | 0                             | 0      |
| TRIM32   | 96.74                 | 99.94               | 100.00                          | 100.00 | 0                             | 0      |
| TTN      | 64.78                 | 64.72               | 99.67                           | 99.59  | 402                           | 497    |
| UBA1     | 87.97                 | 98.47               | 100.00                          | 100.00 | 0                             | 0      |
| VAPB     | 82.07                 | 91.70               | 100.00                          | 100.00 | 0                             | 0      |
| VCP      | 98.44                 | 98.90               | 100.00                          | 100.00 | 0                             | 0      |
| VMA21    | 43.73                 | 68.90               | 100.00                          | 100.00 | 0                             | 0      |
| YARS2    | 88.00                 | 99.99               | 100.00                          | 100.00 | 0                             | 0      |

**Notes:**

<sup>1</sup>Average per-base coverage for gene. Genes that are not completely callable are highlighted in pink. For more information on how coverage was calculated, please refer to *General Methodology* section.

<sup>2</sup>The percent of sites that are considered callable for this gene. For more information on how callability is determined, please refer to *General Methodology* section.

<sup>3</sup>The average for samples included in the cohort.

<sup>4</sup>The metric for the sample in this report.

## **General Methodology**

### ***Exome Sequencing***

We performed whole exome sequencing on DNA samples at the Broad Institute's Genomics Platform, using Illumina exome capture, 38 Mb baited target, and the Broad's in-solution hybrid selection process. For input DNA we used  $>250$  ng of DNA, at  $>2$  ng/ $\mu$ l. Our exome-sequencing pipeline included sample plating, library preparation (2-plexing of samples per hybridization), hybrid capture, sequencing (76 bp paired reads), sample identification QC check, and data storage. Our hybrid selection libraries cover  $>80\%$  of targets at 20x and a mean target coverage of  $>80x$ . The exome sequencing data was de-multiplexed and each sample's sequence data were aggregated into a single Picard BAM file.

### ***Variant Calling, Filtering and Annotation***

Exome sequencing data was processed through a pipeline based on *Picard*, using base quality score recalibration and local realignment at known indels. We used the *BWA* aligner for mapping reads to the human genome build 37 (hg19). Single Nucleotide Polymorphism (SNPs) and insertions/deletions (indels) were jointly called across all samples using *Genome Analysis Toolkit (GATK)* HaplotypeCaller package version 3.1. Default filters were applied to SNP and indel calls using the GATK Variant Quality Score Recalibration (VQSR) approach. Lastly, the variants were annotated using *Variant Effect Predictor (VEP)*.

### ***Ancestry Inference***

A set of  $\sim 5,800$  common SNPs was used to perform principal component analysis (PCA) with 1000 genomes samples and ancestry was inferred based on distance to nearest cluster formed by Exome Aggregation Consortium (ExAC) samples. This analysis is able to identify individuals with a European, South Asian, East Asian, African or Latino ancestry.

### ***Gender Inference***

The gender of the individual was inferred using the fraction of heterozygous calls amongst a set of common variant on chromosome X. In addition, the Y chromosome coverage normalized to chromosome 20 coverage was also used to infer gender.

### ***Gene coverage***

The coding bases (including 10 bp flanking region) of the candidate genes was determined using the Gencode v19 regions. The per base coverage of each sample was determined using *samtools depth*. Only reads with mapping quality (MQ)  $\geq 20$  and bases with base quality (BQ)  $\geq 10$  were included in the coverage metric. Lastly, bases with from overlapping reads were only counted once. A site with coverage  $>6$  was considered callable.

### ***Analysis***

The variant call set was uploaded on to *xBrowse* and an analysis limited to the candidate gene list was performed using the various inheritance patterns. The main report contains variants restricted to nonsense, frameshift, essential splice site and missense variants and filtered on variant site and genotype quality. The appendix listing each gene contains all variants discovered regardless of annotation and quality.

### ***Exome Aggregation Consortium (ExAC) data set***

The Exome Aggregation Consortium (ExAC) is a coalition of investigators seeking to aggregate and harmonize exome sequencing data from a wide variety of large-scale sequencing projects, and to make summary data available for the wider scientific community. The data set contains  $>60,000$  unrelated individuals sequenced as part of various disease-specific and population genetic studies. The allele frequencies are summarized into the major ancestries European, Non-Finnish European, South Asian, East Asian, African and Latino. For more details please visit <http://exac.broadinstitute.org/about>

## ***ClinVar Database***

ClinVar is a freely accessible, public archive of reports of the relationships among human variations and phenotypes, with supporting evidence. The level of confidence in the accuracy of variation calls and assertions of clinical significance depends in large part on the supporting evidence and is variable for the variants included in ClinVar. A review status (stars) is also assigned by ClinVar to each variant, to indicate the level of supporting evidence.

| Stars | Review Status                                                                                                                        |
|-------|--------------------------------------------------------------------------------------------------------------------------------------|
| None  | Either conflicting interpretations (in which case the independent values are enumerated), or no submitter provided an interpretation |
| 1     | One submitter provided an interpretation (classified by single submitter)                                                            |
| 2     | Two or more submitters provided the same interpretation (classified by multiple submitters)                                          |
| 3     | Reviewed by expert panel                                                                                                             |
| 4     | Practice guideline (reviewed by professional society)                                                                                |

## ***Tools/Resources***

| Tool/Resource                       | Link                                                                                                                      |
|-------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| BWA                                 | <a href="https://github.com/lh3/bwa">https://github.com/lh3/bwa</a>                                                       |
| Picard                              | <a href="https://github.com/broadinstitute/picard">https://github.com/broadinstitute/picard</a>                           |
| Genome Analysis Toolkit (GATK)      | <a href="https://www.broadinstitute.org/gatk">https://www.broadinstitute.org/gatk</a>                                     |
| samtools                            | <a href="https://github.com/samtools/samtools">https://github.com/samtools/samtools</a>                                   |
| Gencode v19                         | <a href="http://www.gencodegenes.org/releases/19.html">http://www.gencodegenes.org/releases/19.html</a>                   |
| Variant Effect Predictor (VEP)      | <a href="http://www.ensembl.org/info/docs/tools/vep/index.html">http://www.ensembl.org/info/docs/tools/vep/index.html</a> |
| xBrowse                             | <a href="https://xbrowse.broadinstitute.org">https://xbrowse.broadinstitute.org</a>                                       |
| Exome Aggregation Consortium (ExAC) | <a href="http://exac.broadinstitute.org">http://exac.broadinstitute.org</a>                                               |
| ClinVar                             | <a href="http://www.ncbi.nlm.nih.gov/clinvar">http://www.ncbi.nlm.nih.gov/clinvar</a>                                     |

**Supplementary Material 4 .**  
Tables.

**Suppl. Table 1.** Percentage hydrogen bonds occupancies of the residues of Asp81 and Gly81 in the trajectories of wild-type and Asp81Gly mutant structures, respectively, of ANO5.

|       | Hydrogen bond occupancy [%] with: |          |          |          |
|-------|-----------------------------------|----------|----------|----------|
|       | Arg78                             | Tyr652   | Gln79    | Gln256   |
| Asp81 | 100.0 (A)*                        | 15.8 (A) | 0.66 (A) | 0.0      |
| Gly81 | 0.0                               | 0.0      | 27.2 (D) | 24.9 (A) |

\* (D) and (A) stand for the respective residue at position 81 acting as hydrogen bond donor or acceptor, respectively.

**Suppl. Table 2.** Percentage hydrogen bonds occupancies of the residues of Lys132 and Met132 in the trajectories of wild-type and Lys132Met mutant structures, respectively, of ANO5.

|        | Hydrogen bond occupancy [%] with: |
|--------|-----------------------------------|
|        | Leu115                            |
| Lys132 | 55.4 (D)*                         |
| Met132 | 69.8 (D) + 71.3 (A)               |

\* (D) and (A) stand for the respective residue at position 132 acting as hydrogen bond donor or acceptor, respectively.

**Suppl. Table 3.** Percentage hydrophobic interactions occupancies of the residues of Lys132 and Met132 in the trajectories of wild-type and Lys132Met mutant structures, respectively, of ANO5.

|        | Hydrophobic interaction occupancy [%] with: |        |
|--------|---------------------------------------------|--------|
|        | Leu115                                      | Ala135 |
| Lys132 | 24.2                                        | 0.0    |
| Met132 | 85.5                                        | 97.4   |

**Suppl. Table 4.** Percentage hydrogen bonds occupancies of the residues of Ser555 and Ile555 in the trajectories of wild-type and Ser555Ile mutant structures, respectively, of ANO5.

|        | Hydrogen bond occupancy [%] with: |          |          |
|--------|-----------------------------------|----------|----------|
|        | Glu544                            | Glu551   | Lys559   |
| Ser555 | 97.8 (D)*                         | 19.1 (D) | 15.3 (A) |
| Ile555 | 0.0                               | 68.9 (D) | 52.3 (A) |

\* (D) and (A) stand for the respective residue at position 555 acting as hydrogen bond donor or acceptor, respectively.

**Suppl. Table 5.** Percentage hydrophobic interactions occupancies of the residues of Ser555 and Ile555 in the trajectories of wild-type and Ser555Ile mutant structures, respectively, of ANO5.

|        | Hydrophobic interaction occupancy [%] with: |
|--------|---------------------------------------------|
|        | Pro546                                      |
| Ser555 | 0.0                                         |
| Ile555 | 24.2                                        |

**Suppl. Table 6.** Percentage hydrogen bonds occupancies of the residues of Tyr671 and Cys671 in the trajectories of wild-type and Tyr671Cys mutant structures, respectively, of ANO5.

|        | Hydrogen bond occupancy [%] with: |          |          |
|--------|-----------------------------------|----------|----------|
|        | Arg234                            | Gly668   | Glu675   |
| Tyr671 | 89.2 (D)*                         | 33.6 (D) | 34.6 (A) |
| Cys671 | 0.0                               | 32.8 (D) | 0.0      |

\* (D) and (A) stand for the respective residue at position 671 acting as hydrogen bond donor or acceptor, respectively.

**Suppl. Table 7.** Percentage hydrophobic interactions occupancies of the residues of Tyr671 and Cys671 in the trajectories of wild-type and Tyr671Cys mutant structures, respectively, of ANO5.

|        | Hydrophobic interaction occupancy [%] with: |        |
|--------|---------------------------------------------|--------|
|        | Lys233                                      | Met560 |
| Tyr671 | 20.6                                        | 91.2   |
| Cys671 | 3.0                                         | 0.0    |

**Suppl. Table 8.** Percentage hydrogen bonds occupancies of the residues of Arg758 and Cys758 in the trajectories of wild-type and Arg758Cys mutant structures, respectively, of ANO5.

|        | Hydrogen bond occupancy [%] with: |          |         |          |          |          |          |
|--------|-----------------------------------|----------|---------|----------|----------|----------|----------|
|        | Glu816                            | Asn817   | Lys818  | His821   | Asn822   | Met823   | Gln824   |
| Arg758 | 65.8 (D)*                         | 8.9 (D)  | 5.3 (D) | 49.7 (D) | 55.6 (A) | 31.6 (A) | 0.0      |
| Cys758 | 0.1 (D)                           | 19.9 (D) | 0.0     | 0.0      | 0.0      | 0.0      | 78.1 (A) |

\* (D) and (A) stand for the respective residue at position 758 acting as hydrogen bond donor or acceptor, respectively.

**Suppl. Table 9.** Percentage hydrophobic interactions occupancies of the residues of Arg758 and Cys758 in the trajectories of wild-type and Arg758Cys mutant structures, respectively, of ANO5.

|        | Hydrophobic interaction occupancy [%] with: |        |        |        |
|--------|---------------------------------------------|--------|--------|--------|
|        | Ile756                                      | Val760 | Tyr761 | Met823 |
| Arg758 | 99.1                                        | 0.0    | 0.0    | 84.2   |
| Cys758 | 0.0                                         | 60.1   | 71.9   | 0.0    |

**Suppl. Table 10.** Percentage hydrogen bonds occupancies of the residues of His841 and Asp841 in the trajectories of wild-type and His841Asp mutant structures, respectively, of ANO5.

|        | Hydrogen bond occupancy [%] with: |          |          |         |          |
|--------|-----------------------------------|----------|----------|---------|----------|
|        | Arg364                            | Asn366   | Ile837   | Val838  | Leu845   |
| His841 | 20.2 (A)*                         | -        | 18.8 (D) | 3.9 (D) | 29.1 (A) |
| Asp841 | 60.7 (A)                          | 31.5 (A) | 1.8 (D)  | 7.6 (D) | 16.0 (A) |

\* (D) and (A) stand for the respective residue at position 841 acting as hydrogen bond donor or acceptor, respectively.

**Suppl. Table 11.** Percentage hydrophobic interactions occupancies of the residues of His841 and Asp841 in the trajectories of wild-type and His841Asp mutant structures, respectively, of ANO5.

|        | Hydrophobic interaction occupancy [%] with: |        |        |
|--------|---------------------------------------------|--------|--------|
|        | Arg364                                      | Phe844 | Leu845 |
| His841 | 57.2                                        | 28.1   | 87.6   |
| Asp841 | -                                           | -      | -      |

**Suppl. Table 12.** Hydrophobic contacts between A1, A2, B1 and B2  $\alpha$ -helices present in the average structures from the trajectories of the wild-type ANO5 and its mutants.

| System              | Residue 1        | $\alpha$ -helix | Residue 2 | $\alpha$ -helix | Contact type           |
|---------------------|------------------|-----------------|-----------|-----------------|------------------------|
| <b>1. ANO5</b>      |                  |                 |           |                 |                        |
|                     | Phe 624          | A1              | Trp 1551  | B1              | $\pi$ - $\pi$ stacked  |
|                     | Ala 630, Ile 631 | A1              | Trp 710   | A2              | amide- $\pi$ stacked   |
|                     | Met 618          | A1              | Leu 696   | A2              | alkyl                  |
|                     | Ala 630          | A1              | Val 707   | A2              | alkyl                  |
|                     | Ala 635          | A1              | Ile 1540  | B1              | alkyl                  |
|                     | Ala 635          | A1              | Val 1616  | B2              | alkyl                  |
|                     | Ala 635          | A1              | Val 1620  | B2              | alkyl                  |
|                     | Leu 636          | A1              | Ile 1540  | B1              | alkyl                  |
|                     | Met 618          | A1              | Ala 697   | A2              | alkyl                  |
|                     | Met 1531         | B1              | Leu 1609  | B2              | alkyl                  |
|                     | Met 1531         | B1              | Ala 1606  | B2              | alkyl                  |
|                     | Leu 634          | A1              | Val 1616  | B2              | alkyl                  |
|                     | Ile 631          | A1              | Val 1620  | B2              | alkyl                  |
|                     | Tyr 632          | A1              | Ile 1540  | B1              | $\pi$ -alkyl           |
|                     | Tyr 632          | A1              | Ile 1544  | B1              | $\pi$ -alkyl           |
|                     | Pro 633          | A1              | Trp 710   | A2              | $\pi$ -alkyl           |
|                     | Leu 634          | A1              | Trp 710   | A2              | $\pi$ -alkyl           |
|                     | Lys 628          | A1              | Trp 1551  | B1              | $\pi$ -alkyl           |
|                     | Ile 631          | A1              | Trp 1623  | B2              | $\pi$ -alkyl           |
|                     | Lys 628          | A1              | Trp 1623  | B2              | $\pi$ -alkyl           |
| <b>2. Asp81Gly</b>  |                  |                 |           |                 |                        |
|                     | Met 618          | A1              | Leu 696   | A2              | alkyl                  |
|                     | Ala 635          | A1              | Ile 1544  | B1              | alkyl                  |
|                     | Met 618          | A1              | Ala 697   | A2              | alkyl                  |
|                     | Trp 638          | A1              | Ile 1536  | B1              | $\pi$ -alkyl           |
|                     | Ile 627          | A1              | Trp 1551  | B1              | $\pi$ -alkyl           |
| <b>3. Lys132Met</b> |                  |                 |           |                 |                        |
|                     | Trp 639          | A1              | Phe 1537  | B1              | $\pi$ - $\pi$ T-shaped |
|                     | Met 618          | A1              | Leu 696   | A2              | alkyl                  |
|                     | Ile 631          | A1              | Ile 1540  | B1              | alkyl                  |

|                     |          |    |          |    |                        |
|---------------------|----------|----|----------|----|------------------------|
|                     | Ala 635  | A1 | Ile 1536 | B1 | alkyl                  |
|                     | Met 618  | A1 | Ala 693  | A2 | alkyl                  |
|                     | Met 618  | A1 | Ala 697  | A2 | alkyl                  |
|                     | Ile 623  | A1 | Val 703  | A2 | alkyl                  |
|                     | Met 1531 | B1 | Leu 1609 | B2 | alkyl                  |
|                     | Ile 627  | A1 | Lys 1541 | B1 | alkyl                  |
|                     | Ala 1543 | B1 | Val 1620 | B2 | alkyl                  |
|                     | Ala 1543 | B1 | Lys 1624 | B2 | alkyl                  |
|                     | Ile 623  | A1 | Ala 1548 | B1 | alkyl                  |
|                     | Met 1531 | B1 | Ala 1606 | B2 | alkyl                  |
|                     | Met 1531 | B1 | Ala 1610 | B2 | alkyl                  |
|                     | Trp 638  | A1 | Ile 1536 | B1 | $\pi$ -alkyl           |
|                     | Ala 630  | A1 | Trp 710  | A2 | $\pi$ -alkyl           |
|                     | Ile 631  | A1 | Trp710   | A2 | $\pi$ -alkyl           |
| <b>4. Ser555Ile</b> |          |    |          |    |                        |
|                     | Trp 639  | A1 | Ile 1536 | B1 | $\pi$ -sigma           |
|                     | Phe 624  | A1 | Trp 1552 | B1 | $\pi$ - $\pi$ T-shaped |
|                     | Met 618  | A1 | Leu 696  | A2 | alkyl                  |
|                     | Leu 634  | A1 | Ile 1540 | B1 | alkyl                  |
|                     | Ala 635  | A1 | Ile 1536 | B1 | alkyl                  |
|                     | Met 618  | A1 | Ala 693  | A2 | alkyl                  |
|                     | Met 618  | A1 | Ala 697  | A2 | alkyl                  |
|                     | Met 1531 | B1 | Leu 1609 | B2 | alkyl                  |
|                     | Ala 1543 | B1 | Val 1620 | B2 | alkyl                  |
|                     | Ile 627  | A1 | Ala 1548 | B1 | alkyl                  |
|                     | Leu 1547 | B1 | Lys 1624 | B2 | alkyl                  |
|                     | Trp 638  | A1 | Ile 1540 | B1 | $\pi$ -alkyl           |
|                     | Trp 639  | A1 | Ile 1536 | B1 | $\pi$ -alkyl           |
|                     | Ile 623  | A1 | Trp 1552 | B1 | $\pi$ -alkyl           |
|                     | Ile 627  | A1 | Trp 1552 | B1 | $\pi$ -alkyl           |
| <b>5. Tyr671Cys</b> |          |    |          |    |                        |
|                     | Met 618  | A1 | Leu 696  | A2 | alkyl                  |
|                     | Ala 630  | A1 | Val 707  | A2 | alkyl                  |
|                     | Ile 631  | A1 | Ile 1540 | B1 | alkyl                  |
|                     | Ile 631  | A1 | Ile 1544 | B1 | alkyl                  |

|                     |                  |    |          |    |                       |
|---------------------|------------------|----|----------|----|-----------------------|
|                     | Leu 634          | A1 | Ile 1536 | B1 | alkyl                 |
|                     | Leu 634          | A1 | Ile 1540 | B1 | alkyl                 |
|                     | Ala 635          | A1 | Ile 1540 | B1 | alkyl                 |
|                     | Met 618          | A1 | Ala 697  | A2 | alkyl                 |
|                     | Ala 1543         | B1 | Lys 1624 | B2 | alkyl                 |
|                     | Ile 631          | A1 | Ala 1548 | B1 | alkyl                 |
|                     | Leu 1527         | B1 | Ala 1606 | B2 | alkyl                 |
|                     | Ala 630          | A1 | Trp 710  | A2 | $\pi$ -alkyl          |
|                     | Leu 634          | A1 | Trp 710  | A2 | $\pi$ -alkyl          |
|                     | Ile 699          | A2 | Trp1551  | B1 | $\pi$ -alkyl          |
| <b>6. Arg758Cys</b> |                  |    |          |    |                       |
|                     | Ile 623          | A1 | Leu 1547 | B1 | alkyl                 |
|                     | Ala 635          | A1 | Ile 1536 | B1 | alkyl                 |
|                     | Met 1531         | B1 | Leu 1609 | B2 | alkyl                 |
|                     | Ile 623          | A1 | Ala 1548 | B1 | alkyl                 |
| <b>7. His841Asp</b> |                  |    |          |    |                       |
|                     | Trp 639          | A1 | Phe 1537 | B1 | $\pi$ - $\pi$ stacked |
|                     | Trp 638, Trp 639 | A1 | Phe 1537 | B1 | amide- $\pi$ stacked  |
|                     | Met 618          | A1 | Leu 696  | A2 | alkyl                 |
|                     | Ile 627          | A1 | Leu 1547 | B1 | alkyl                 |
|                     | Lys 628          | A1 | Ile 1544 | B1 | alkyl                 |
|                     | Ala 635          | A1 | Ile 1540 | B1 | alkyl                 |
|                     | Met 618          | A1 | Ala 693  | A2 | alkyl                 |
|                     | Met 1531         | B1 | Leu 1609 | B2 | alkyl                 |
|                     | Met 1531         | B1 | Ala 1610 | B2 | alkyl                 |
|                     | Phe 624          | A1 | Ile 1544 | B1 | $\pi$ -alkyl          |
|                     | Trp 638          | A1 | Ile 1536 | B1 | $\pi$ -alkyl          |
|                     | Ile 616          | A1 | Trp 1551 | B1 | $\pi$ -alkyl          |
|                     | Ile 623          | A1 | Trp 1551 | B1 | $\pi$ -alkyl          |
|                     | Leu 1547         | B1 | Trp 1623 | B2 | $\pi$ -alkyl          |

**Suppl. Table 13.** Hydrogen bonds above 10% between central  $\alpha$ -helices from chains A and B present in the trajectories of the wild-type ANO5 and its mutants.

| System    | Acceptor | $\alpha$ -helix | Donor    | $\alpha$ -helix | % in trajectory |
|-----------|----------|-----------------|----------|-----------------|-----------------|
| ANO5      | Ile 1540 | B1              | Tyr 632  | A1              | 71.9            |
| ANO5      | Ile 627  | A1              | Trp 1623 | B2              | 57.4            |
| Asp81Gly  | Asn 1550 | B1              | Asn 626  | A1              | 45.2            |
| His841Asp | Thr 1532 | B1              | Trp 638  | A1              | 31.7            |
| Lys132Met | Thr 1532 | B1              | Trp 638  | A1              | 27.5            |

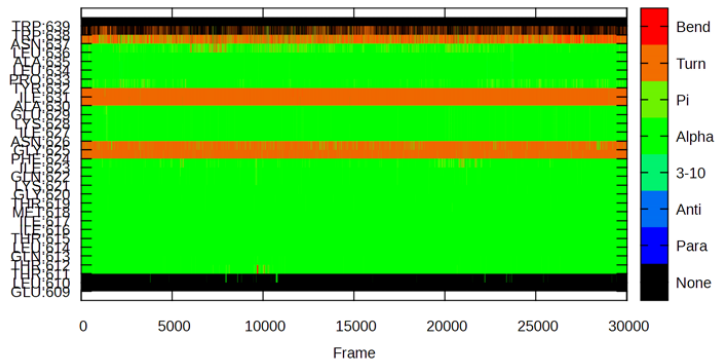
**Jarmula, Lusakowska et al.**

**Supplementary Material 5. Secondary structure assignment.**

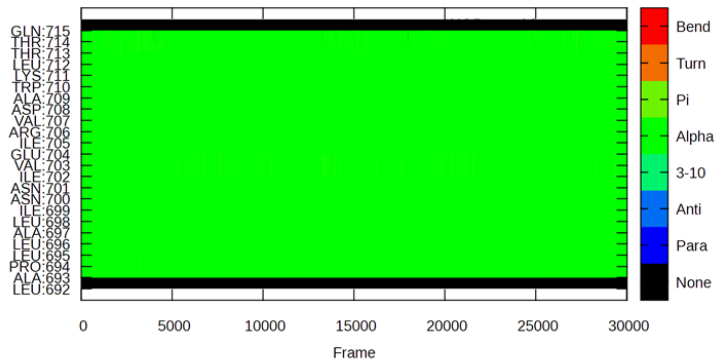
The graphs shown below present evolution of secondary structure assignment of the A1/A2/B1/B2 and 669-684  $\alpha$ -helices in the course of trajectories of wild-type ANO5 and its mutants: Asp81Gly, Lys132Met, Ser555Ile, Tyr671Cys, Arg758Cys and His841Asp. The assignment for  $\alpha$ -helix 669-684 is provided only for the wild-type protein and Tyr671Cys mutant as Tyr671 residue is located within this helix.

## ANO5

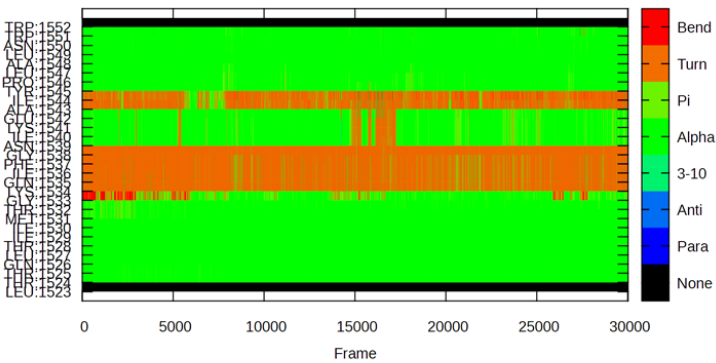
Helix A1



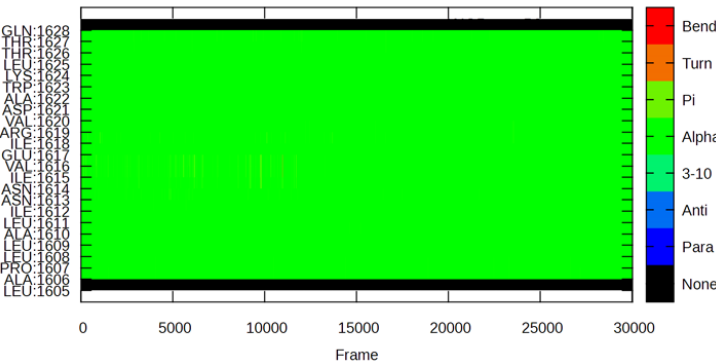
Helix A2



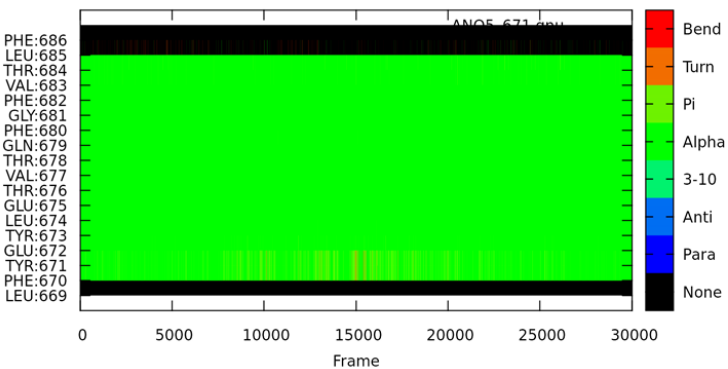
Helix B1



Helix B2

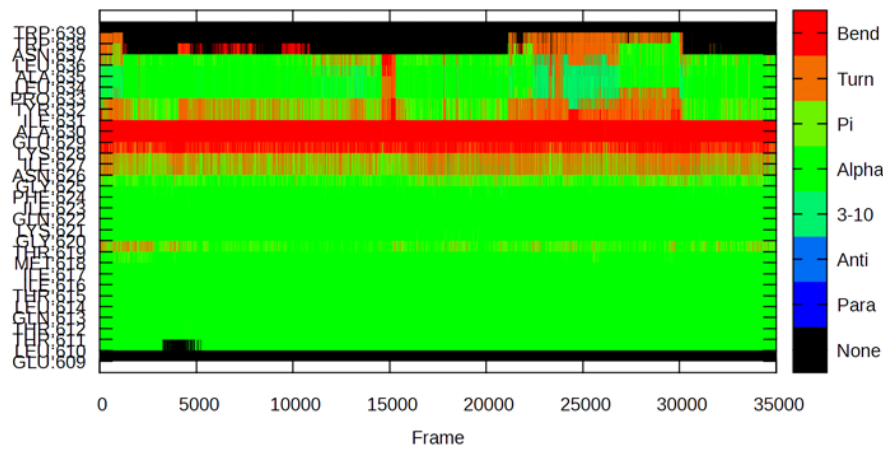


Helix 669-684

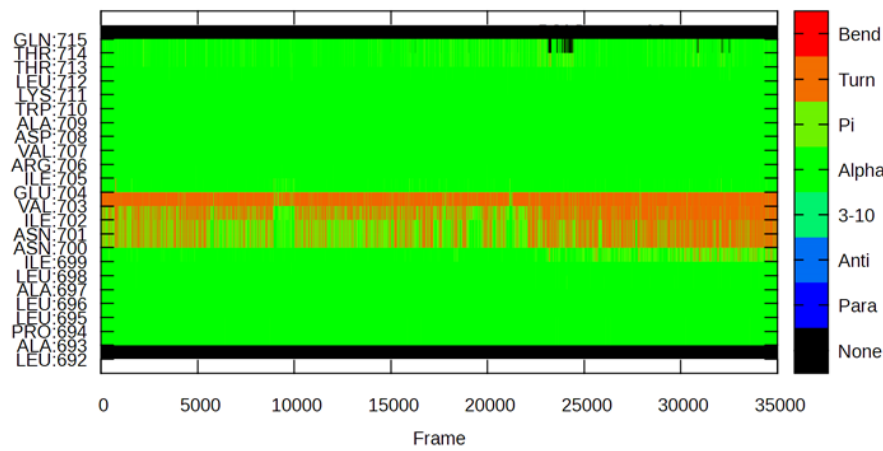


Asp81Gly

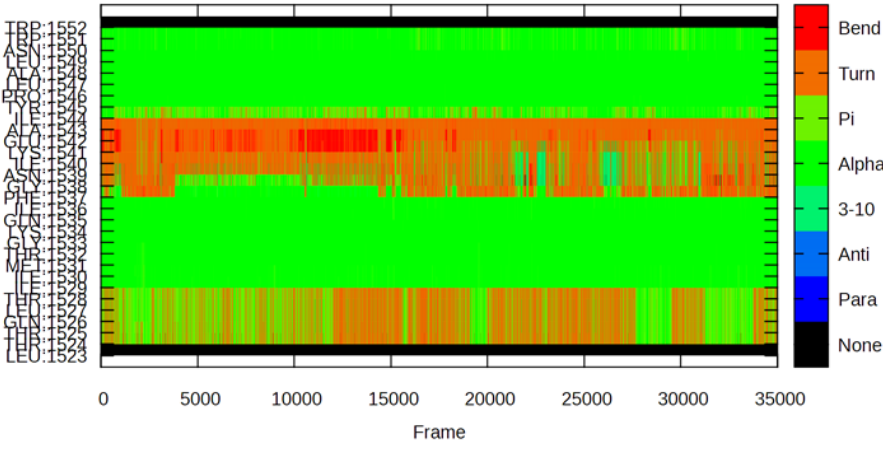
Helix A1



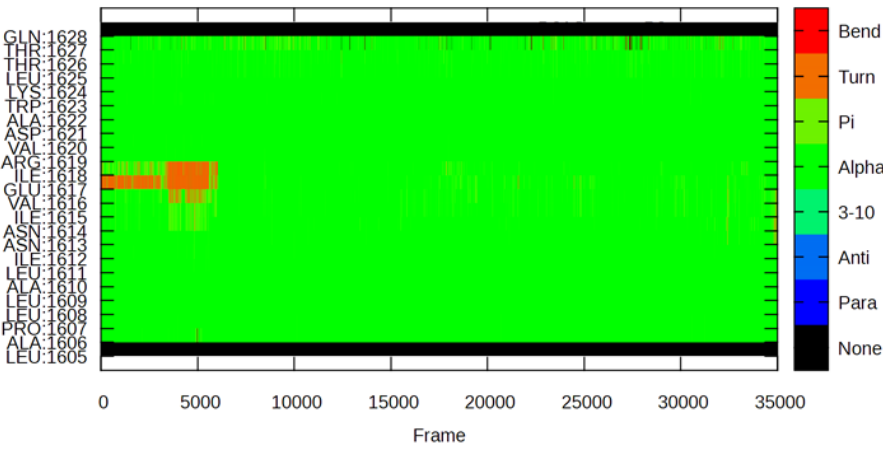
Helix A2



Helix B1

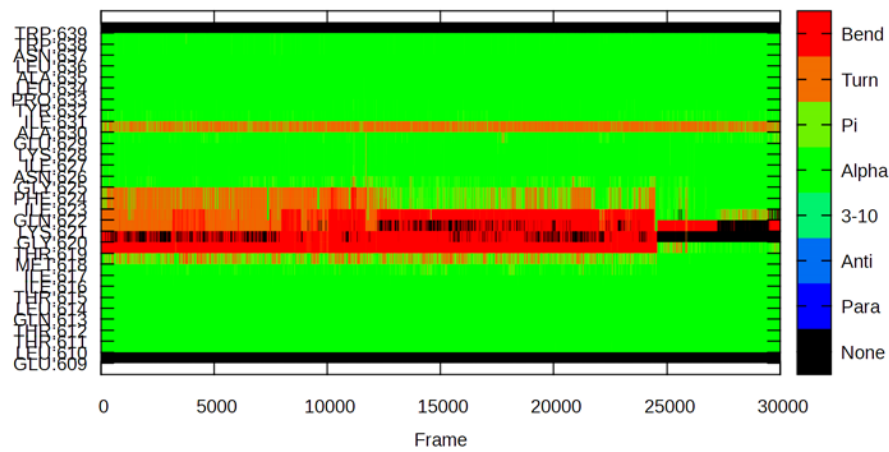


Helix B2

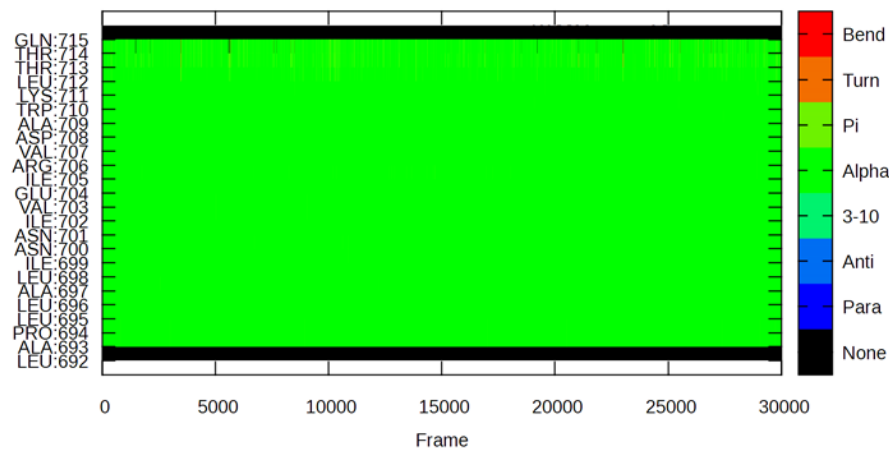


Lys132Met

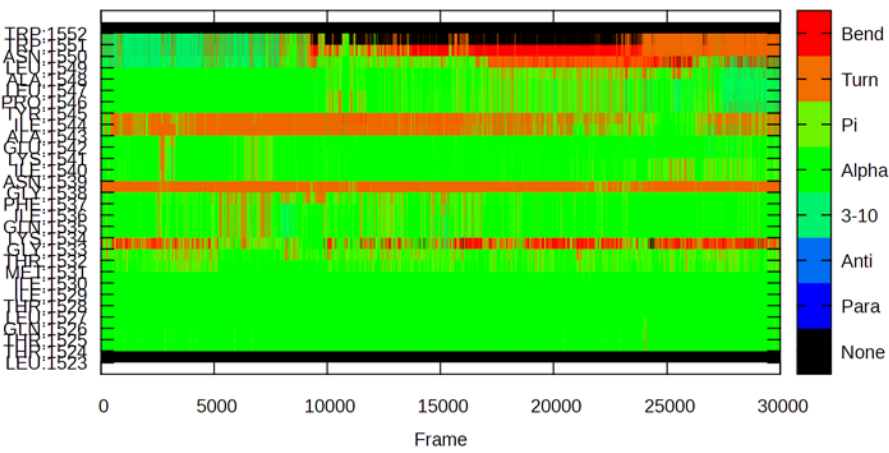
Helix A1



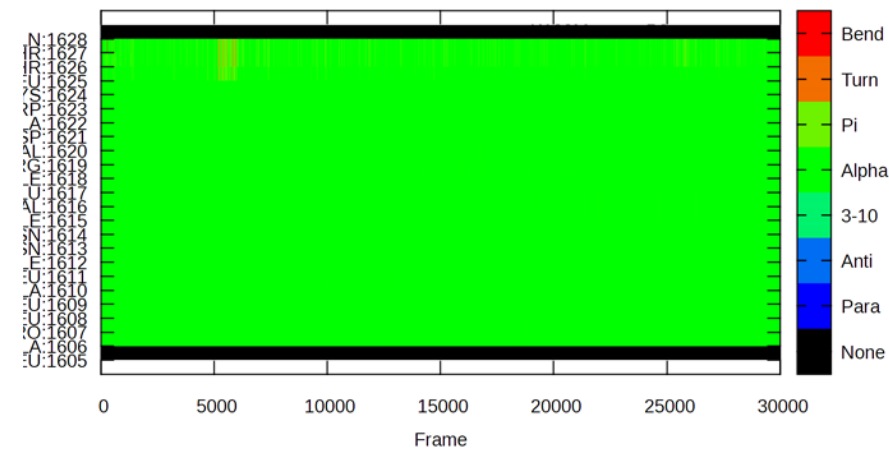
Helix A2



Helix B1

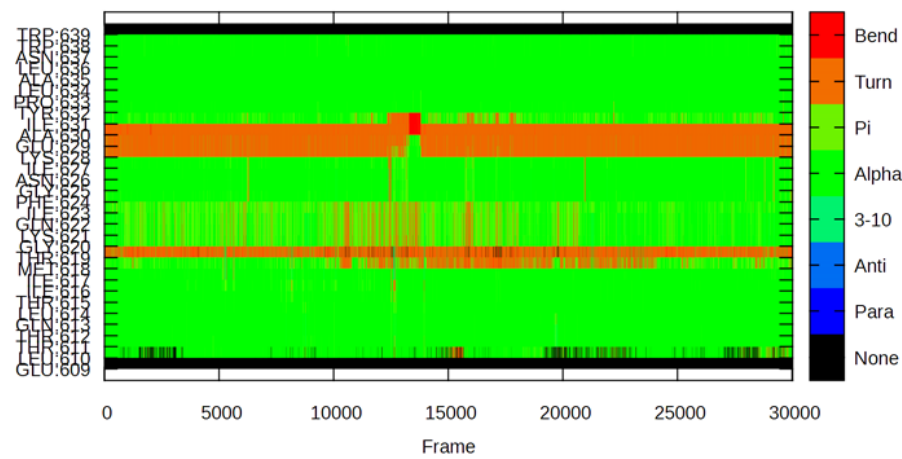


Helix B2

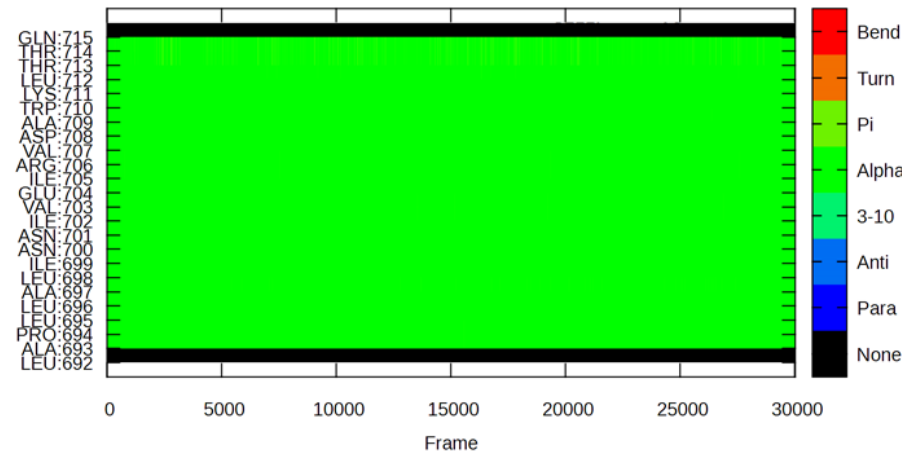


Ser555Ile

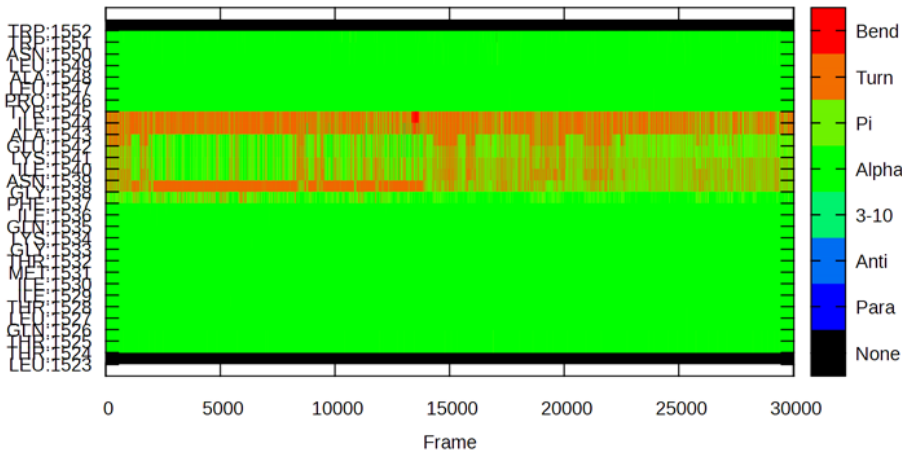
Helix A1



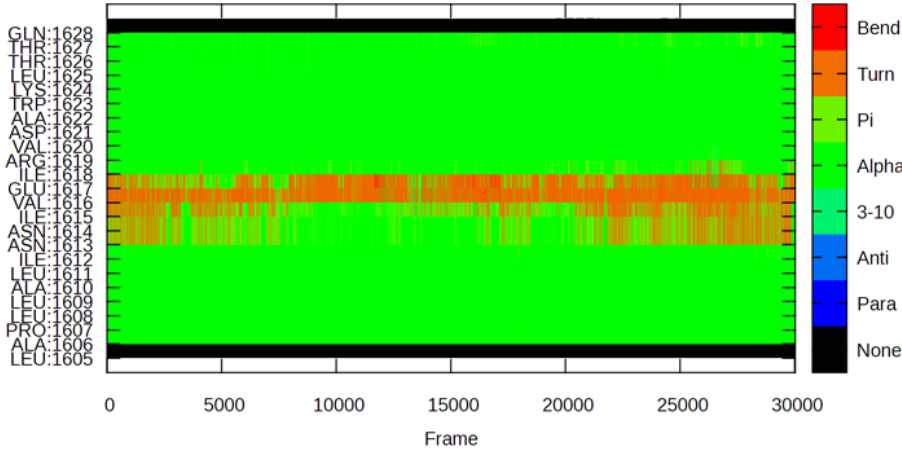
Helix A2



Helix B1

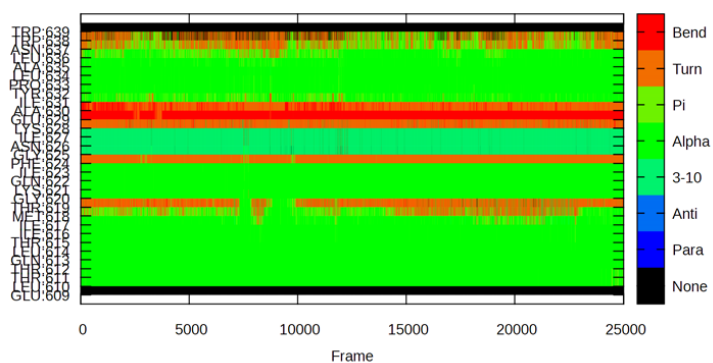


Helix B2

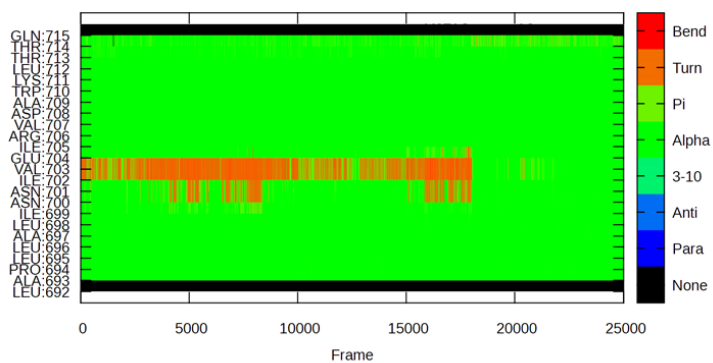


# Tyr671Cys

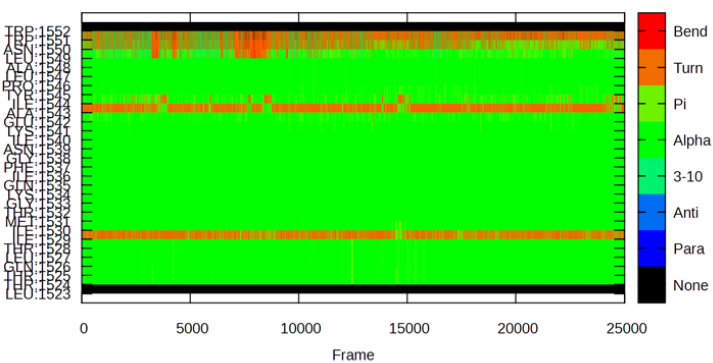
Helix A1



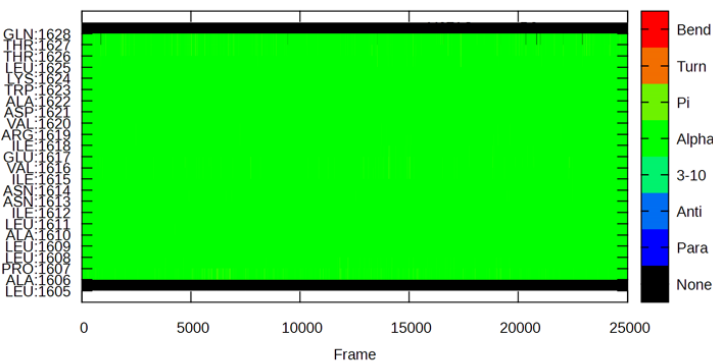
Helix A2



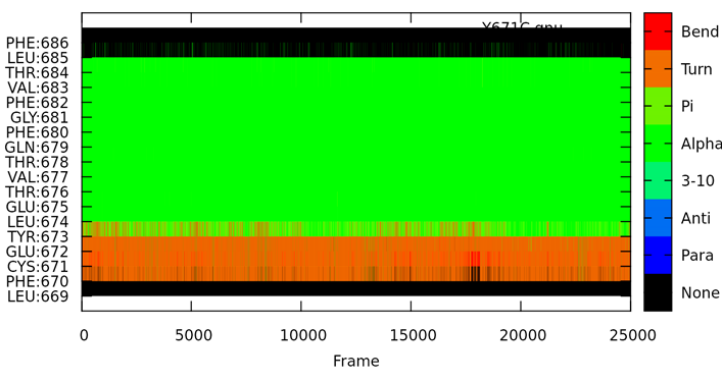
Helix B1



Helix B2

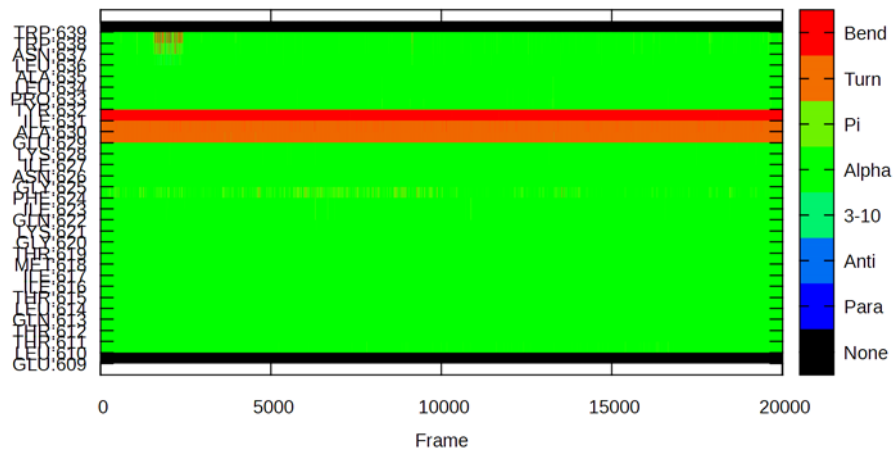


Helix 669-684

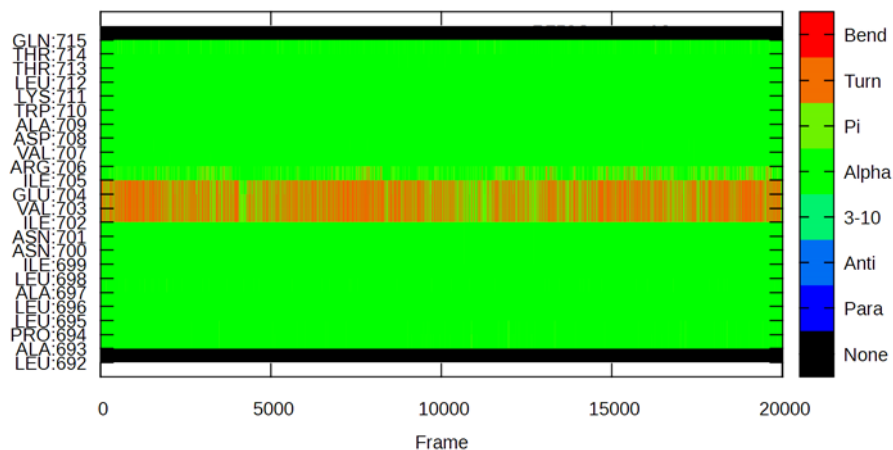


Arg758Cys

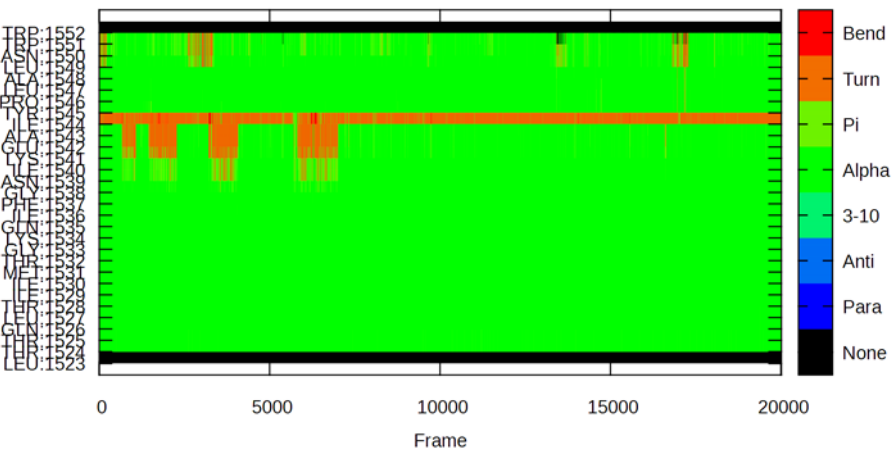
Helix A1



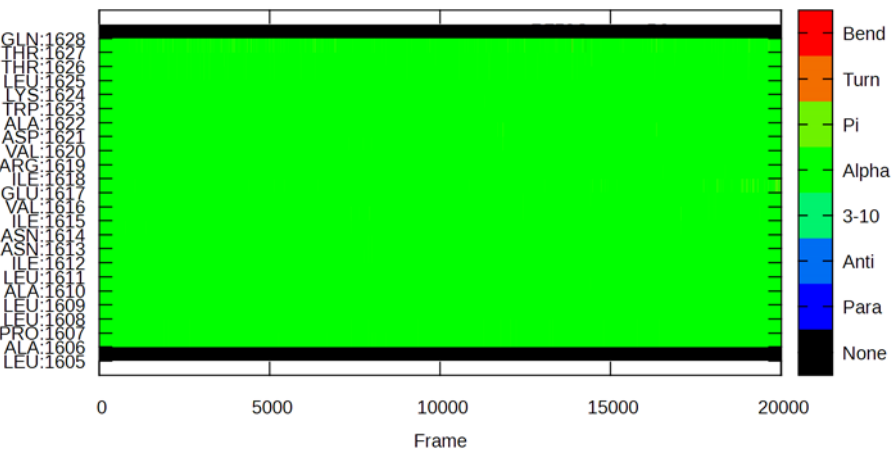
Helix A2



Helix B1

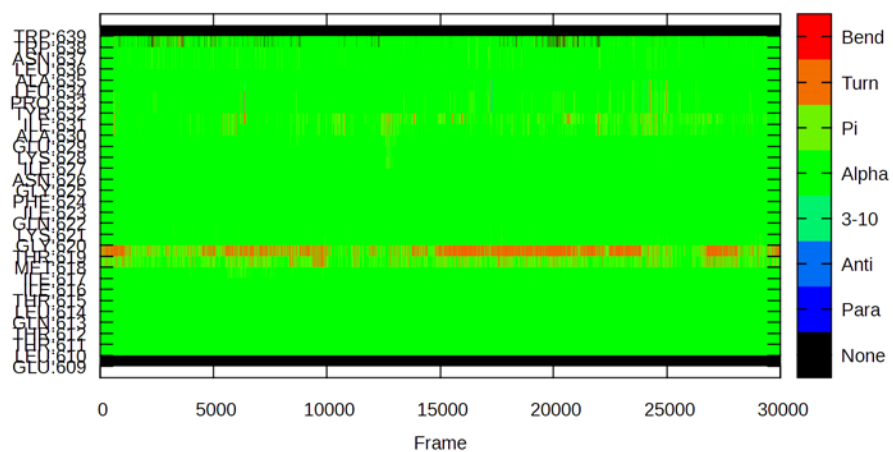


Helix B2

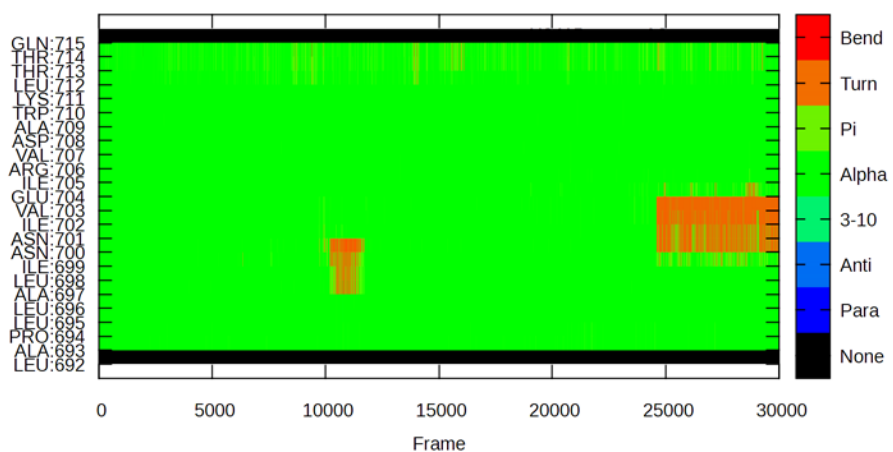


## His841Asp

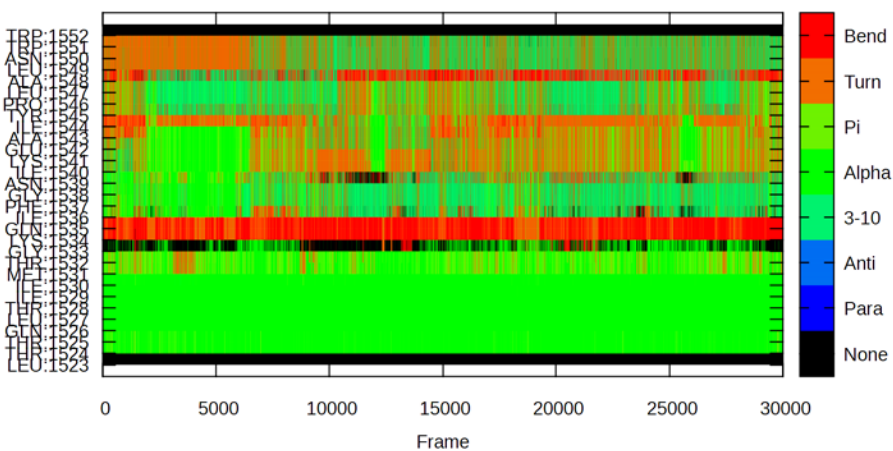
Helix A1



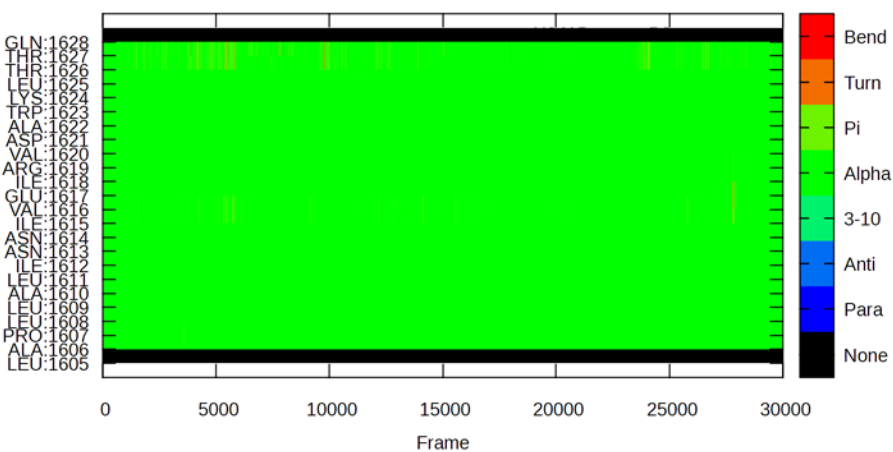
Helix A2



Helix B1



Helix B2



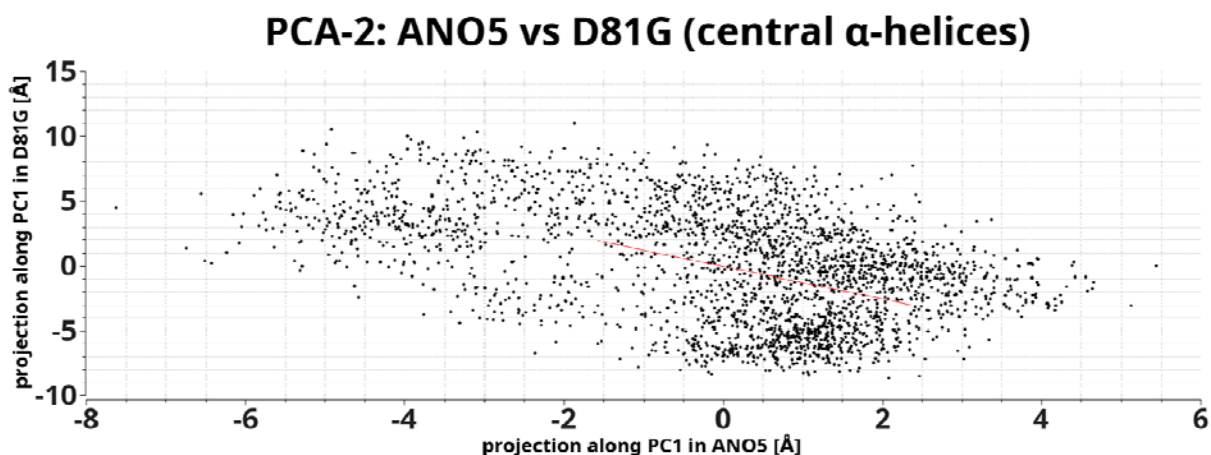
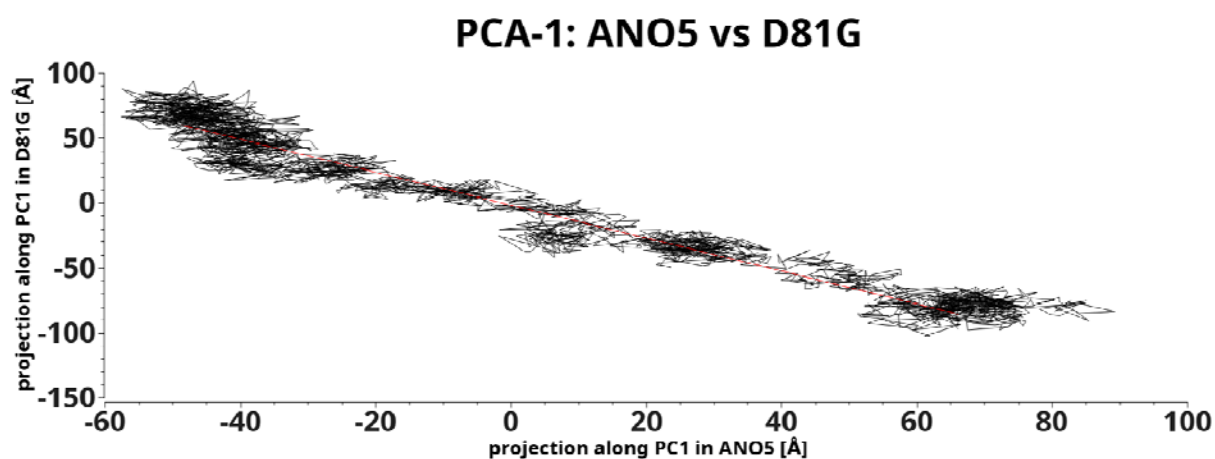
**Jarmula, Lusakowska et al.**

## **Supplementary Material 6.**

Principal Component Analyses (Essential Dynamics).

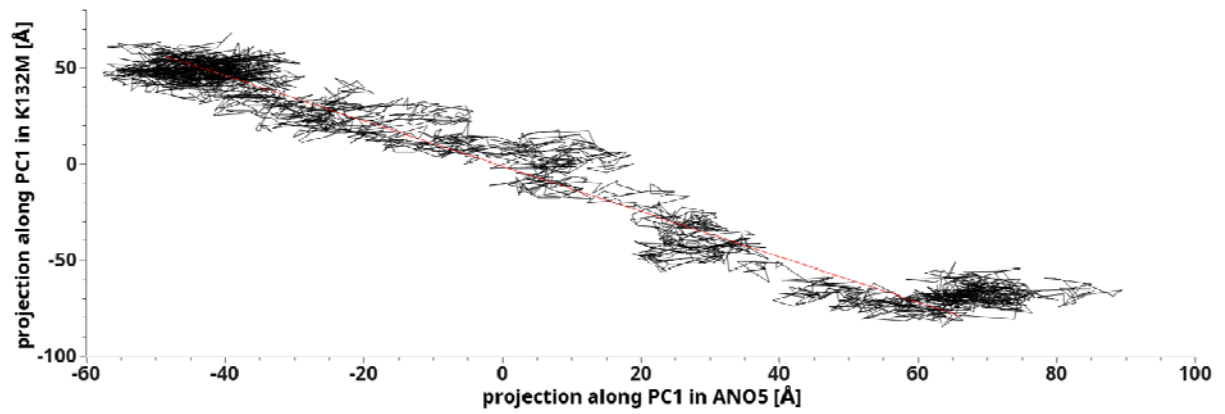
The plots shown below present time development of the projections of trajectories along the first eigenvector (PC1) in PCA-1 and PCA-2 plotted against each other in the wild-type ANO5 versus Asp81Gly/Lys132Met/Ser555Ile/Tyr671Cys/Arg758Cys/His841Asp mutant pairs.

### **ANO5 versus Asp81Gly**

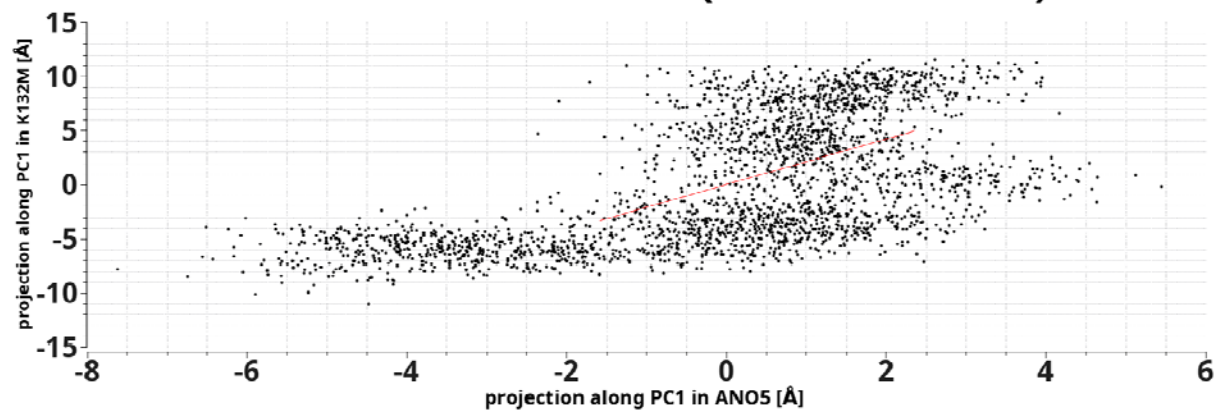


## ANO5 versus Lys132Met

**PCA-1: ANO5 vs K132M**

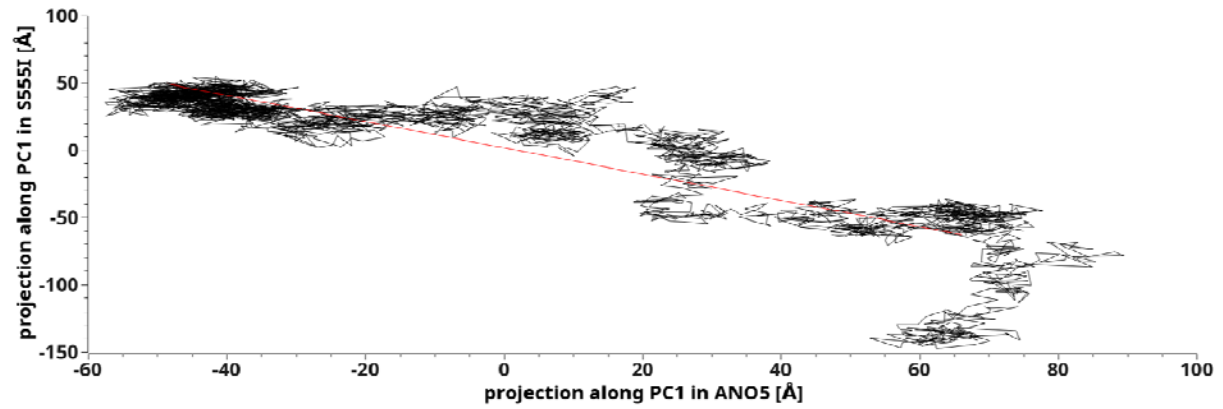


**PCA-2: ANO5 vs K132M (central  $\alpha$ -helices)**

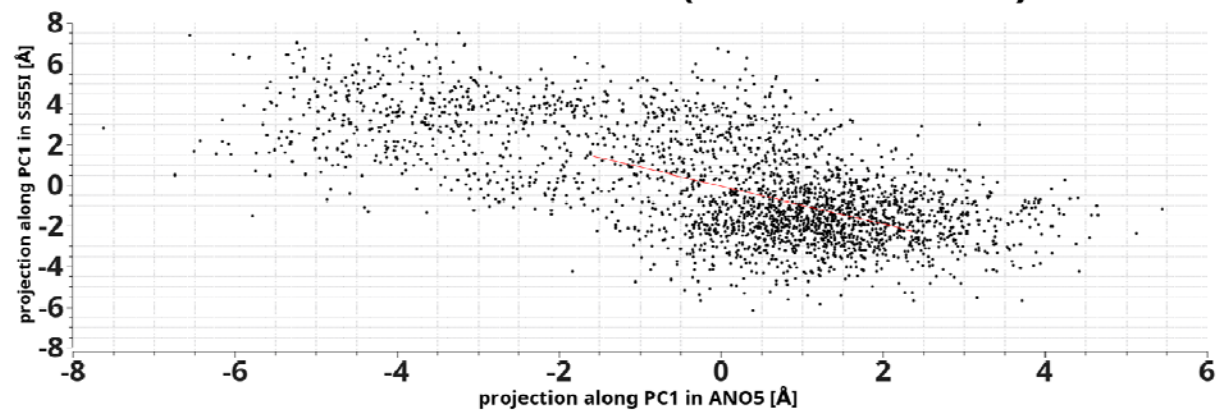


## ANO5 versus Ser555Ile

**PCA-1: ANO5 vs S555I**

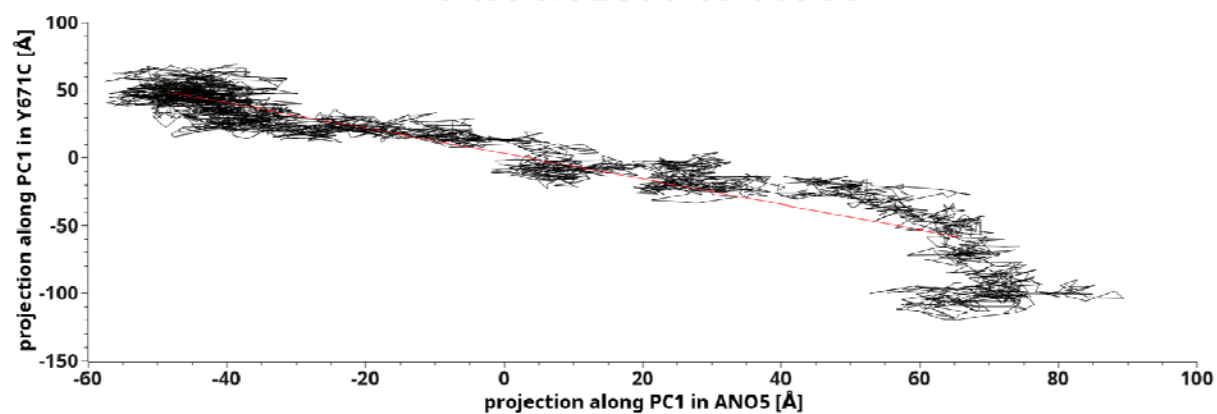


**PCA-2: ANO5 vs S555I (central  $\alpha$ -helices)**

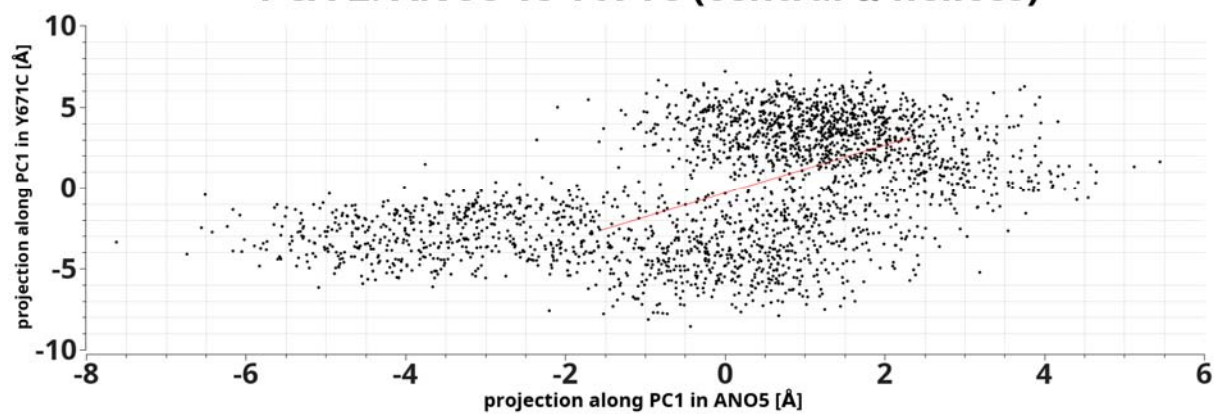


## ANO5 versus Tyr671Cys

**PCA-1: ANO5 vs Y671C**

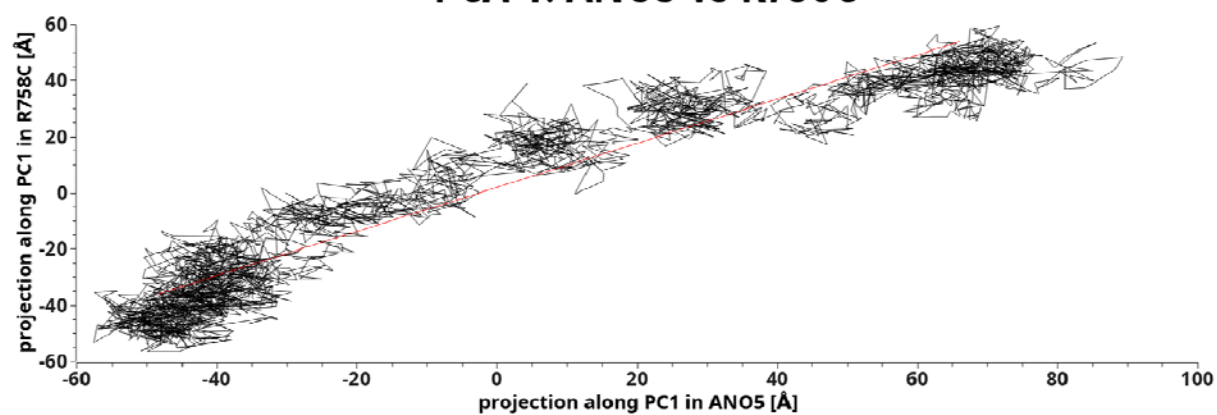


**PCA-2: ANO5 vs Y671C (central  $\alpha$ -helices)**

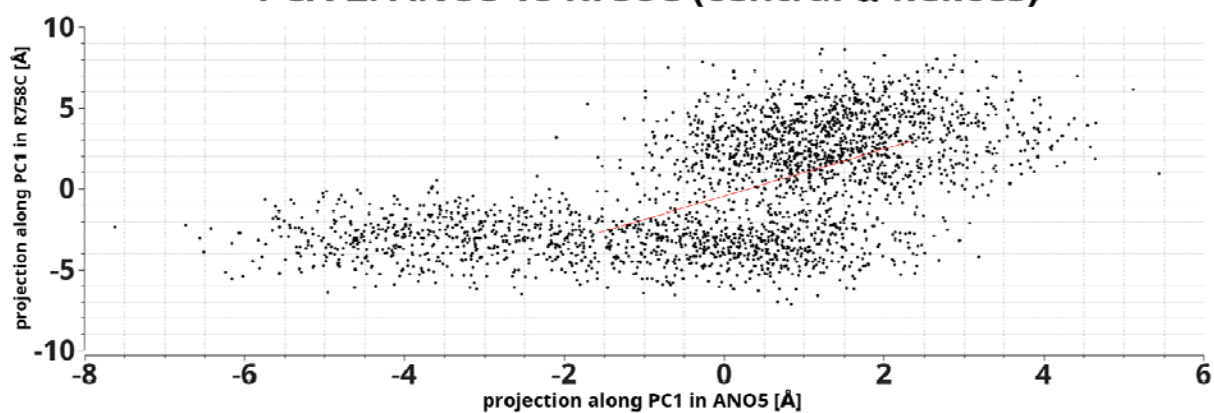


## ANO5 versus Arg758Cys

**PCA-1: ANO5 vs R758C**

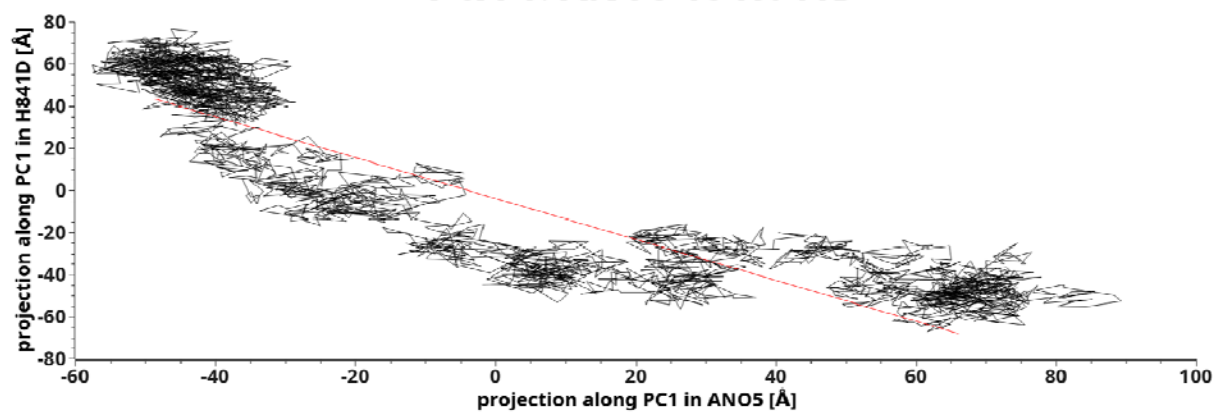


**PCA-2: ANO5 vs R758C (central  $\alpha$ -helices)**

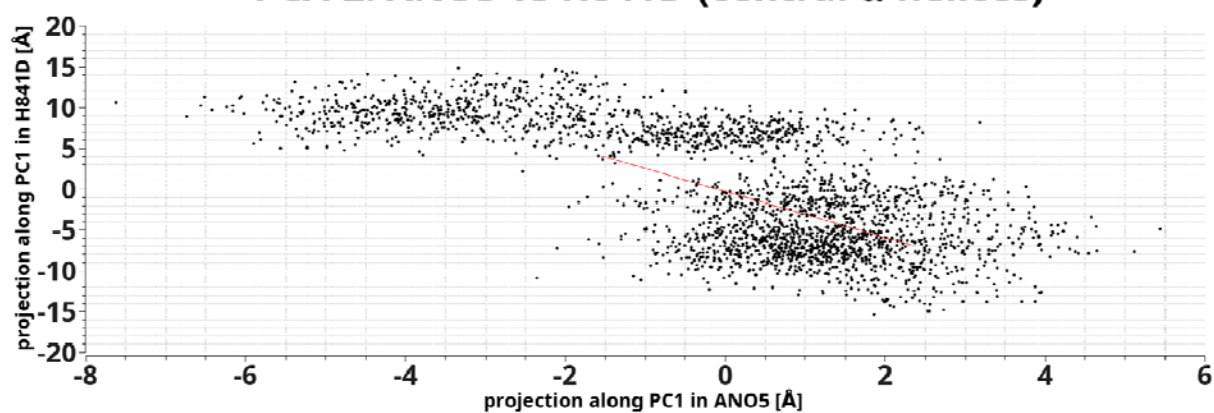


## ANO5 versus His841Asp

**PCA-1: ANO5 vs H841D**



**PCA-2: ANO5 vs H841D (central  $\alpha$ -helices)**



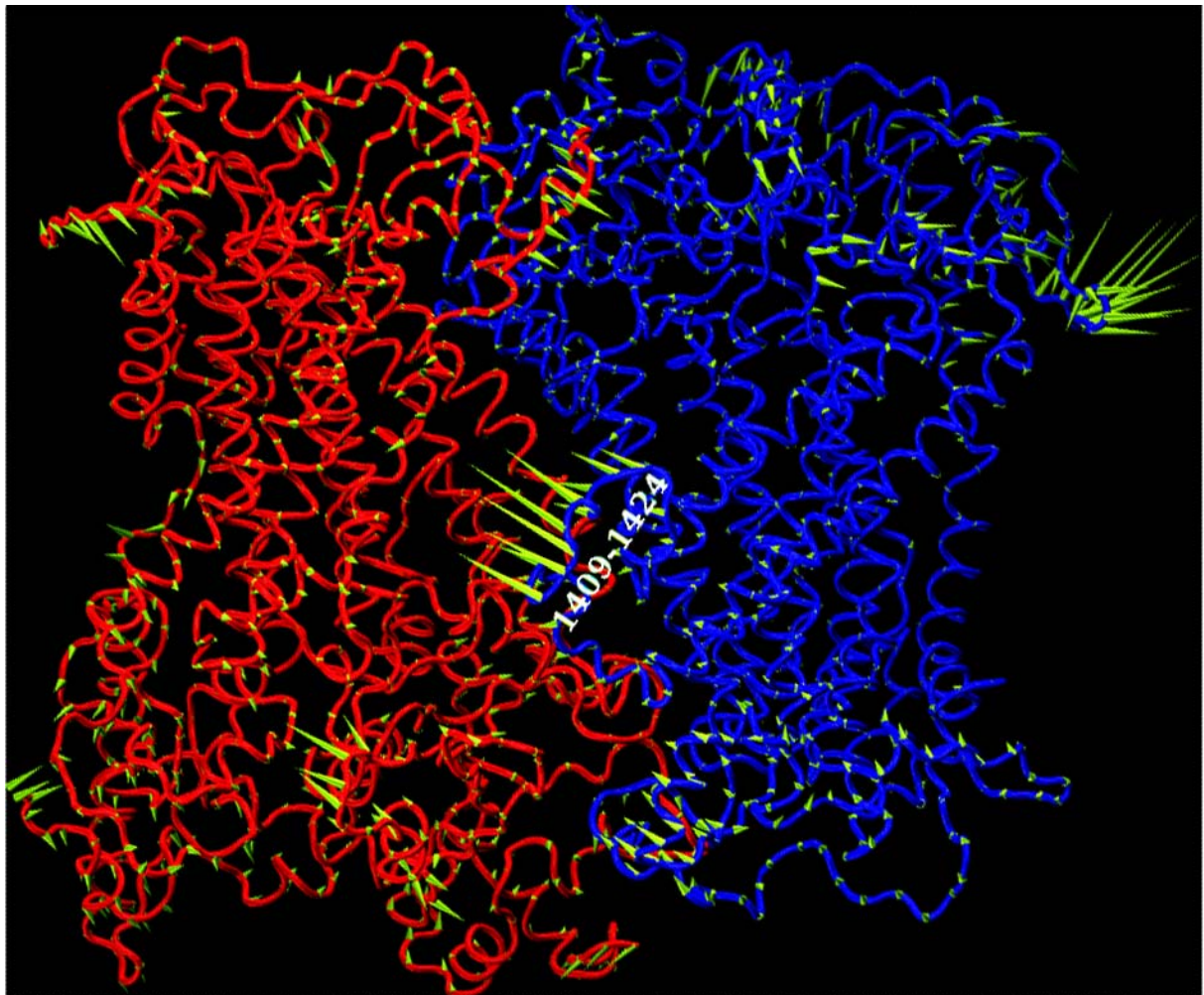
**Jarmula, Lusakowska et al.**

## **Supplementary Material 7.**

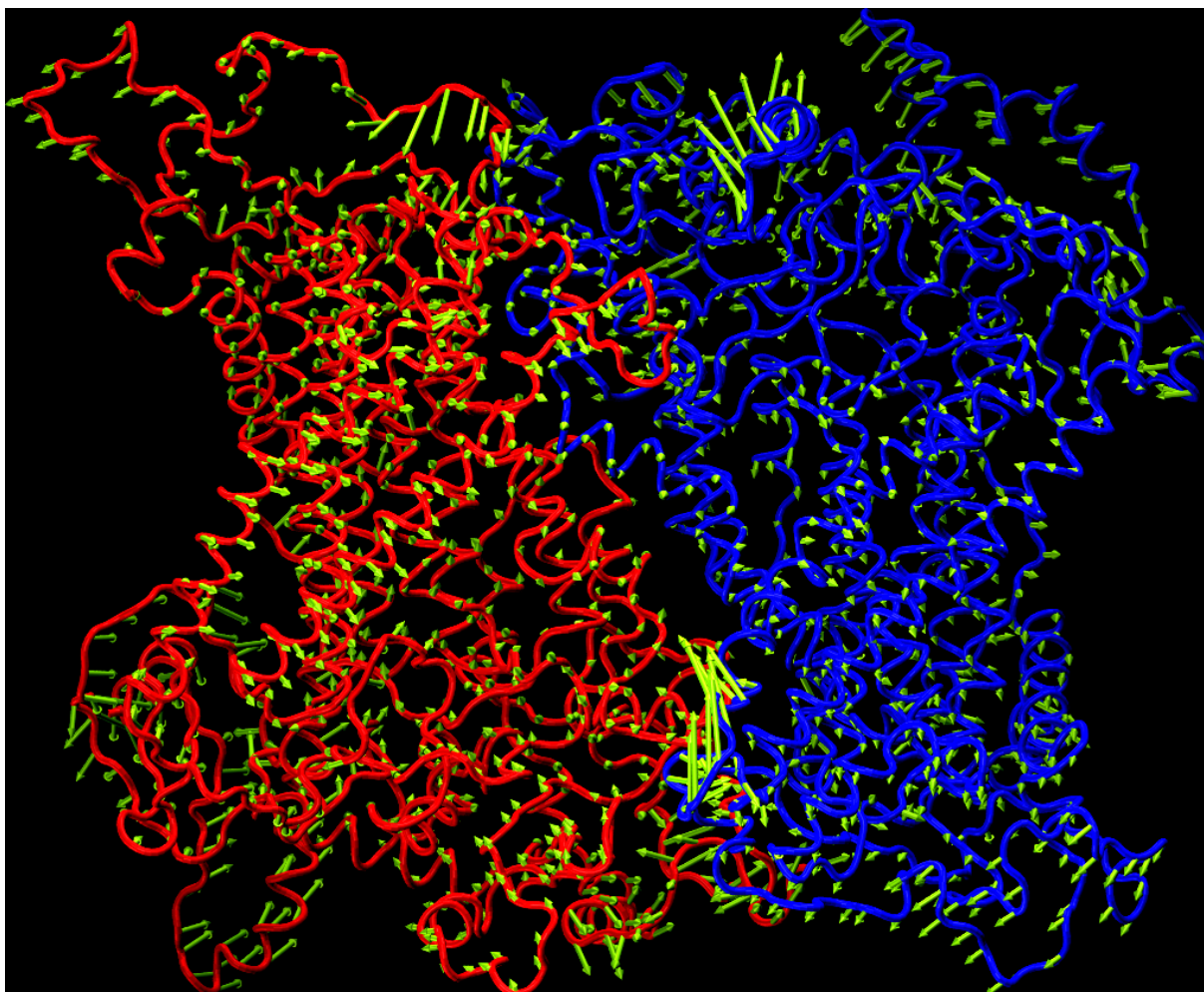
PCA-1 mode-1

The images presented below reflect concerted motions associated with the first eigenvectors (PC1) in PCA-1 for the structures of wild-type ANO5 and its mutants. Chains of subunits A and B are shown as C-alpha tubes and colored red and blue, respectively. The actual frames represent the average structures from the first mode of motion. Motions are represented by green arrows indicating the direction of motion and its amplitude. 1409-1424, the central loop from subunit B.

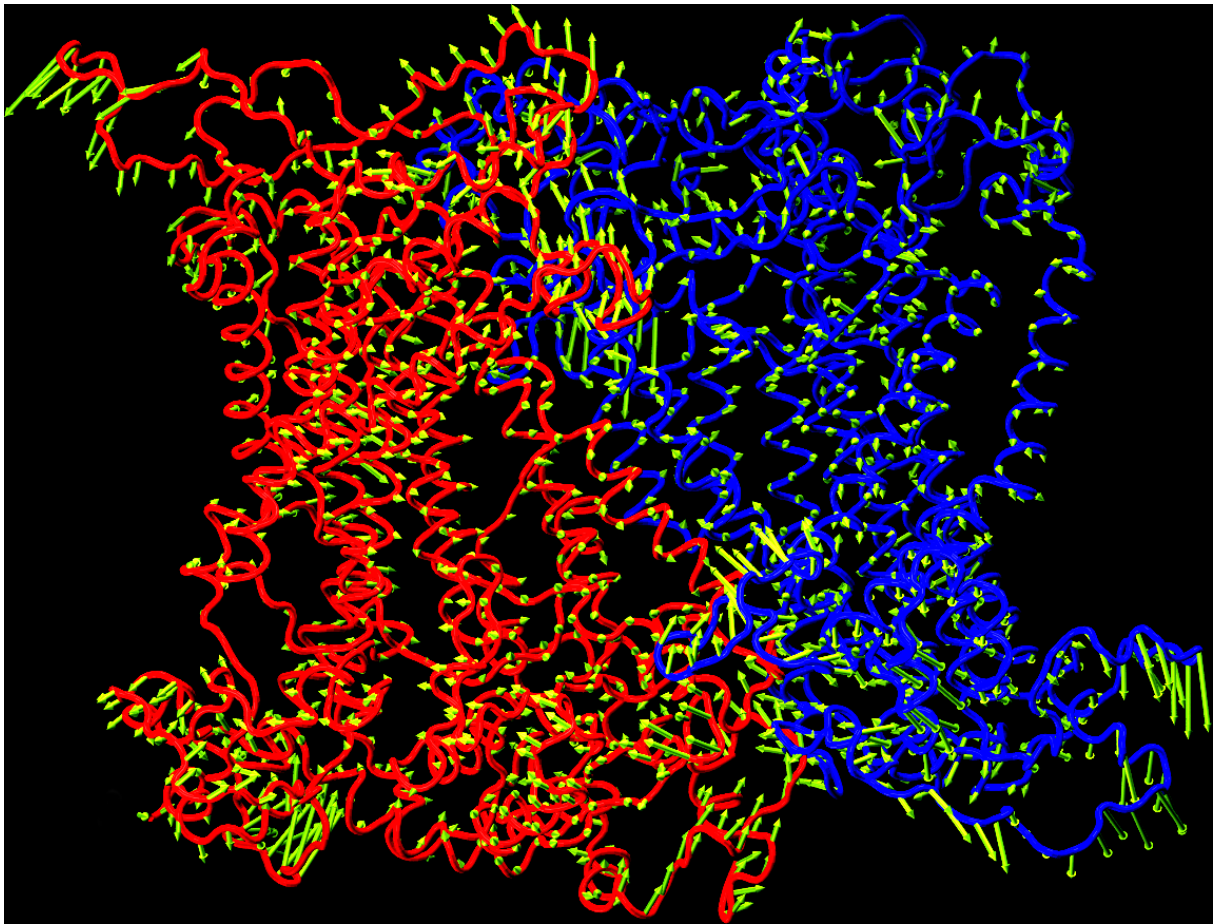
**ANO5**



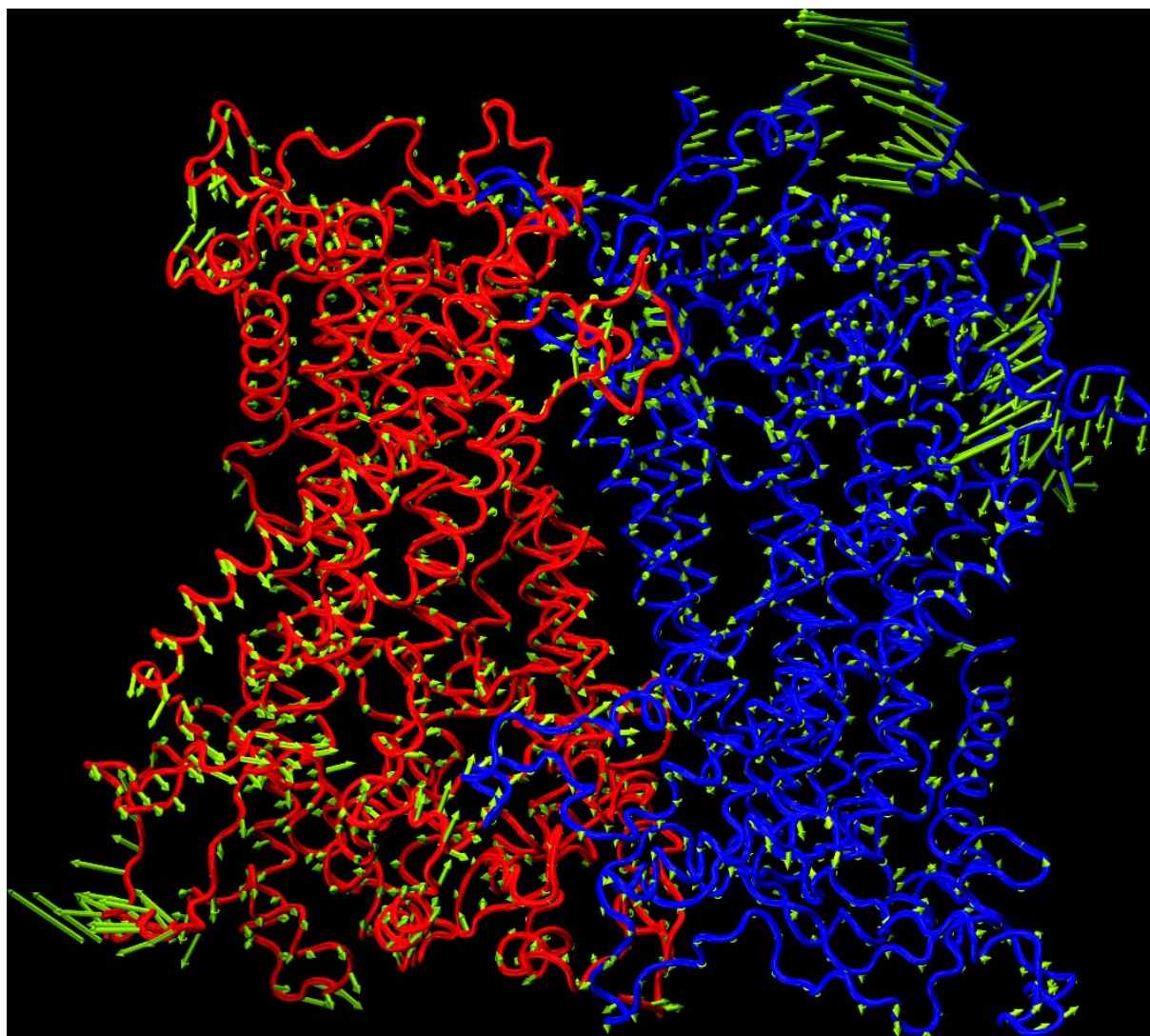
Asp81Gly mutant



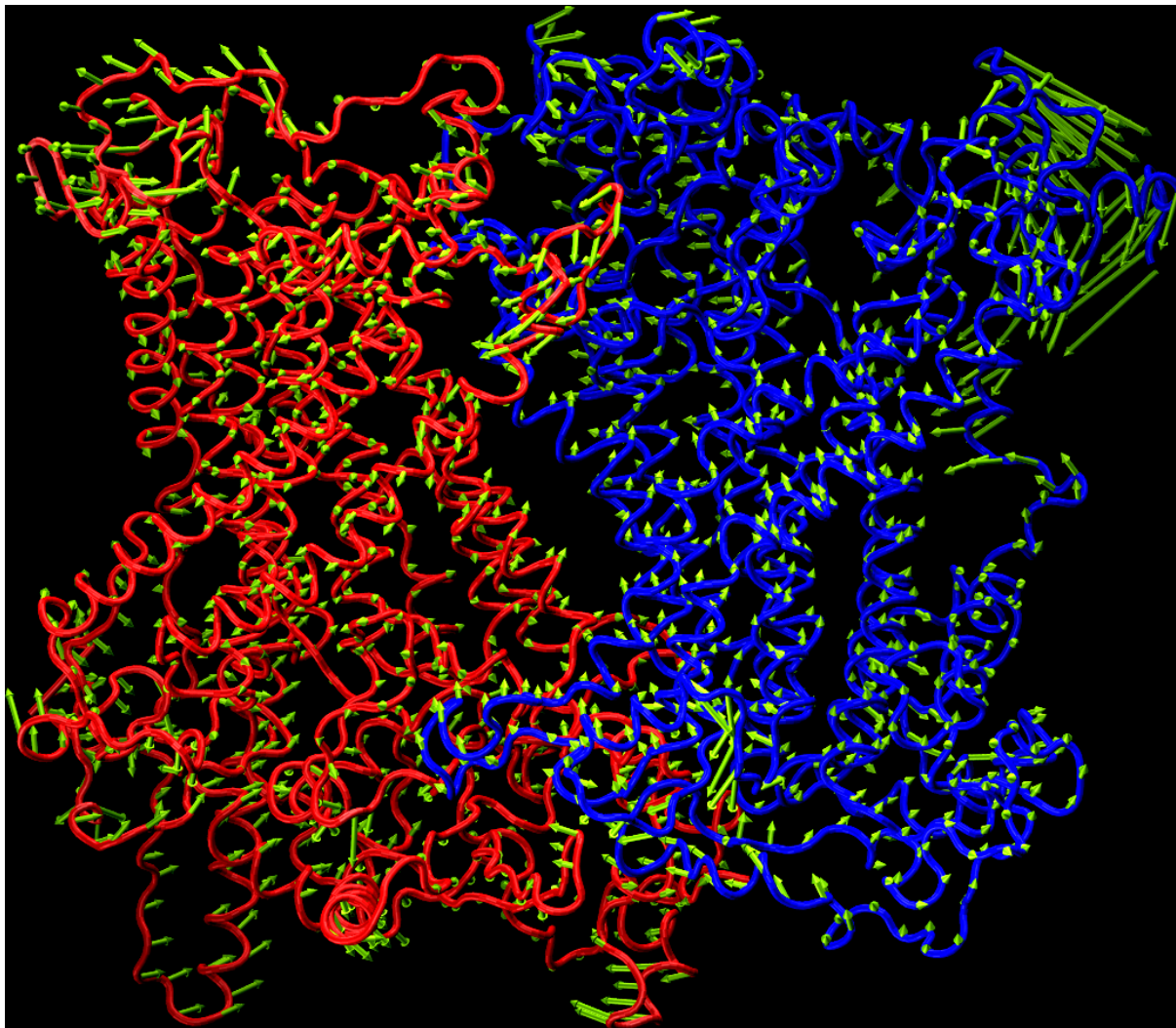
**Lys132Met mutant**



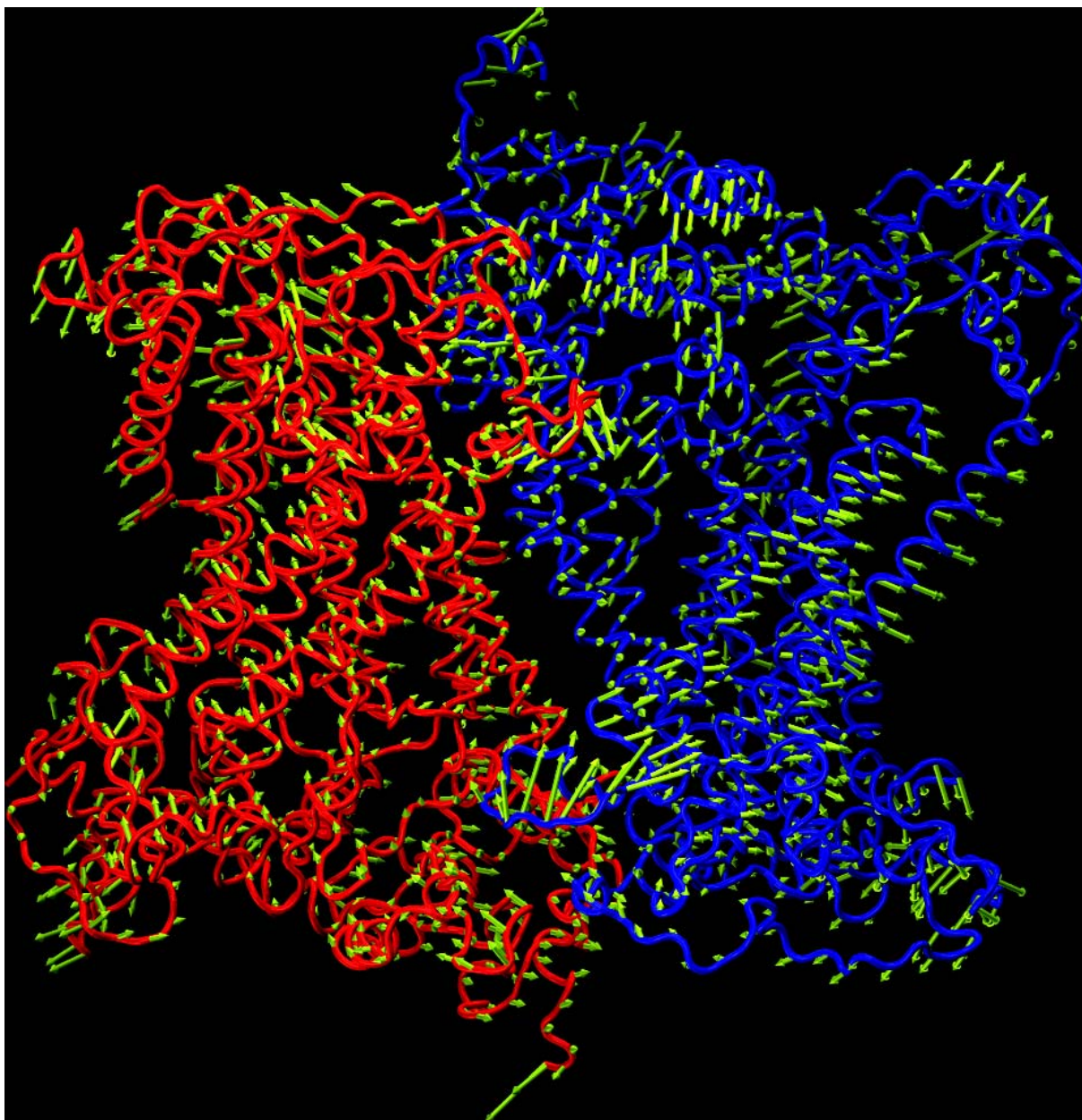
Ser555Ile mutant



## Tyr671Cys mutant



Arg758Cys mutant



His841Asp mutant

